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THE ANATOMY OF OPHIOGLOSSUM PENDULUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 183

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(WITH SIXTEEN FIGURES)

The section OPHIODERMA of the genus *Ophioglossum*, as first separated by PRANTL (**6**), is distinguished from EUOPHIOGLOSSUM by the numerous vascular strands in the base of the petiole, and from CHEIROGLOSSA, which shares this feature, by the occurrence of a single spike on each fertile leaf. As originally described, the section was composed of the single species *O. pendulum* L.; *O. intermedium* Hook. was considered to be a young form of *O. pendulum*, and was included with that species. In 1904, BOWER (**2**) described the new species *O. simplex* Ridley, from Sumatra, and from an examination of its vascular system showed that it is a member of this section. He further reestablished the species *O. intermedium* Hook. on the basis of its intermediate position between *O. pendulum* and *O. simplex*.

Of the three species constituting the section, *O. pendulum* is best known. BOWER (**1**) has described the development of the fertile spike and sporangia, and in a later paper (**2**) has given a brief account of its anatomy. This paper deals merely with the vascular system of the leaf and its insertion upon that of the stem. The investigation here described has been undertaken with a view to supplementing this account of the anatomy of the species.

Material

A large number of entire plants was collected by Dr. CHARLES J. CHAMBERLAIN in November 1911, in Northern Australia. The exact locality is near Babinda, in the Cairns district of Queensland, at a distance of about 25 miles from the coast. Occasional clusters of plants were found near the ground, but more usually they occurred at a considerable height, up to 30 meters. In almost all cases they were growing in the masses of humus accumulated by large plants of *Polypodium rigidulum; Lycopodium phlegmaria* was a common associate. Plants of this species were also observed on Stradbroke Island, near Brisbane, but no collections were made. The groups of plants here were at a less distance from the ground; they were commonly attached to plants of *Platycerium alcicorne* and *P. grande*.

In the material secured at Babinda, the largest leaves had attained a length of about 1.5 meters. However, some of the plants on Stradbroke Island had leaves nearly 2 meters long, and Mr. WM. GIBSON, a collector familiar with that region, reported a specimen with leaves measuring 2.7 meters in length. The largest spikes seen measured 30 cm. in length and 1.2 cm. in greatest width. Many cases of branching or lobing of the spikes were observed; probably one-tenth of the specimens showed some variation from the simple form.

This supply of entire plants was supplemented by a large number of rhizomes, root tips, buds, and spikes collected by Dr. W. J. G. LAND on the island of Tutuila, Samoa, in October 1912. These plants, together with many ferns and occasional plants of *Lycopodium phlegmaria*, occurred in the root masses of *Asplenium nidus*, at a height of 2–10 meters; a large cluster is shown in fig. 1. The leaves reached a length of 2 meters; branching of the leaf was not uncommon, but branching or lobing of the spike was relatively rare. The largest spike collected measured 51 cm. in length and 1.3 cm. in width; sporangia occurred along 45 cm. of its length. Several spikes more than 40 cm. in length were found.

The Australian material was preserved in 6 per cent formalin; that secured in Samoa was killed in 50 per cent alcohol and 6 per cent formalin. Even the largest rhizomes cut readily in paraffin

at 10–25 μ, and complete series were easily obtained. The safranin-anilin blue stain combination gave the best results.

FIG. 1.—*Ophioglossum pendulum*, epiphytic upon *Citrus* sp.; Tutuila, Samoa; photograph by Dr. W. J. G. LAND.

The root

The root stele at the point of attachment to the stem cylinder varies from diarch to pentarch; triarch and tetarch arrangements of the xylem are commonest. In passing from the base of the root toward the tip, the number of protoxylem points frequently increases by the splitting of one of the original protoxylem strands; in one case, the disappearance of a protoxylem strand was noted. In a few cases only, a hexarch arrangement was found; these roots were all very large. In general there is a definite relation between the size of the root and the number of protoxylem strands.

Each phloem mass is a layer 1 or 2 cells in thickness, separated from the xylem by 3–5 layers of parenchyma. It is easily distinguished by the somewhat thickened walls and the conspicuous "proteid granules" adhering to the walls. In the apical region of the root, the protophloem can usually be distinguished considerably in advance of the protoxylem. When a protoxylem strand splits, the phloem between the branches of the split strand connects with the phloem masses on either side.

There is no pericyclic layer; the phloem abuts directly upon the endodermis (fig. 10), which can be readily distinguished by the suberized band upon the radial walls; it resembles the endodermis of *Botrychium virginianum* in all particulars. Two or three cells of parenchyma separate the protoxylem from the endodermis.

Two general regions of the cortex about equal in thickness are distinguishable. In the inner region, the cell walls are heavily thickened by cellulose; simple circular or oval pits occur. In the outer region, the thickening of the cell walls is much less and gives the reactions of pectin compounds. Intercellular spaces of considerable size occur in the inner region; in the outer region, these are much smaller and become filled by the substance which constitutes the thickening of the cell walls. The endophytic fungus which occurs commonly in the roots is confined to the outer region.

The outer walls of the surface cells are remarkably thickened, as shown in fig. 2. The thickening material is not cellulose, but gives the reaction of pectin compounds. The thickening may continue until the lumen of the cell is almost filled; even in these con-

ditions the protoplasm remains very active. When cells of the epidermal layer are killed, the outer walls of the cells immediately below begin to thicken in this way. This may occur in any of the cells of the outer region of the cortex upon destruction of the overlying cells.

FIG. 2.—Epidermal cell of root; ×236.

Roots originate in the meristematic region of the stem by the formation of an apical cell in the first layer of cells outside the phloem. Fig. 3 represents this origin as seen in a longitudinal section of the stem. As shown, differentiation of the phloem has proceeded to within a few cells of the position of the root meristem. The growth of the root proceeds by the segmentation of the apical cell, which is tetrahedral in form; in slowly growing roots the segmentation is irregular, and a definite root cap cannot be distinguished. The first xylem elements are smaller in cross-section than those formed later; they are true tracheids with walls reticulately thickened. In a few cases of more rapidly growing roots, the thickening of the walls approaches the annular marking. This character of the protoxylem indicates the slow growth of the roots. The lignification proceeds slowly toward the center of the stele, and xylem parenchyma is often found in the center of the stele of large roots at a distance of 3–4 cm. from the tips. There is no secondary thickening of the xylem mass. In a few cases a medullated

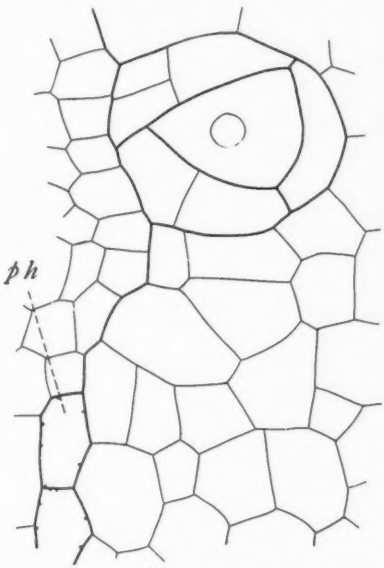

FIG. 3.—Origin of root, as shown in longitudinal section of a bud: *ph*, phloem; ×236.

condition occurred, as shown in fig. 4; the pith is not xylem parenchyma, but is composed of cells resembling those of the cortex; the opening through the xylem always occurs, but the endodermis is not interrupted. This occurs only in roots upon which buds have developed stems; the open portion of the xylem mass is upon the upper side of the root. This condition persists for only a short distance from the bud toward the tip of the root; the gap soon closes and a solid xylem mass is again formed. This peculiar root stele form is directly related to the formation of the stem stele from buds upon the roots, as described later.

The branching of the root is strictly monopodial. The lateral roots grow at an angle of 60–90° to the main root; two or three branch roots often develop at one point. These are not restricted in their growth, as in *Helminthostachys*, but may reach as great length as the primary roots.

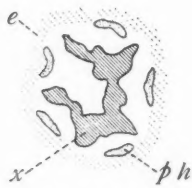

FIG. 4.—Medullated root stele: *x*, xylem; *ph*, phloem; *e*, endodermis; ×37.

Immediately before a branch is given off, the phloem of the main stele spreads laterally between the endodermis and the protoxylem, and forms a continuous sheath about the xylem. A strand of xylem from each of two poles of the main root runs into the branch; hence the lateral roots at the point of attachment are diarch. The phloem sheath surrounds the xylem of the branch in the same way that it does that of the main root; the endodermis also connects without a gap. After the branch has separated, the phloem between the protoxylem and the endodermis disappears, and the usual arrangement of the stelar elements is restored.

In all cases, the branch stele is diarch at the point of attachment, but it may arise from a single pole of the main root. The protoxylem strands of the branch soon split, and the usual triarch or tetrarch condition is established. In one case, a branch arises from a large tetrarch root in which lignification has just begun; each pole is represented by three or four tracheids only. Two distinct strands of xylem, connecting with two poles of the main root, enter the branch. Each xylem strand is surrounded by a sheath

of phloem and endodermis. At a little distance, the endodermal sheaths come into contact, then fuse to form a single sheath; the phloem shows the same behavior. Xylem parenchyma then appears between the two protoxylem strands and the usual diarch condition of the branch stele is established.

The bud

The vegetative reproduction of the species is accomplished by buds upon the roots, as in *O. vulgatum*. As many as three buds were observed upon a single root; the second leaf of the oldest bud was just appearing, while the youngest bud was a mere swelling about 5 mm. from the root tip. It is almost certain that the plants of a colony have all developed from a single plant by this method of vegetative propagation; every rhizome examined in which the base is intact shows by its connection with a root that it has developed from a bud; fig. 5 represents such a case.

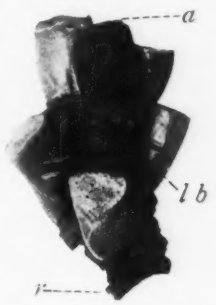

FIG. 5.—A rhizome showing radial character: *a*, stem tip; *lb*, base of a decayed leaf; *r*, parent root; ×1.

ROSTOWZEW (**7**) has described the development of the bud in *O. vulgatum*. In the second segment of the apical cell of the root a new apical cell arises; this produces all stem tissues. The root apical cell is retarded for a time, but finally resumes growth. This results in the formation of a bud with its axis approximately at right angles to the parent root.

The development of the bud has been examined in *O. pendulum;* it agrees in all important points with that of *O. vulgatum*. The retardation of the root growth is usually less; the bud axis often diverges less abruptly from that of the root. Two roots usually develop upon a bud before the first leaf is formed. The details of the vascular connection between the stem developed from a bud and the parent root will be described later.

The stem

The largest rhizome of the Australian material measures 0.7 cm. in diameter and 2.5 cm. in length. There are 18 leaves still attached and the bases of 5 others are evident. All the rhizomes, with the exception of the very youngest, give evidence of having grown in a horizontal position; field observations by Dr. CHAMBERLAIN confirm this. The leaf bases, however, are not restricted to the dorsal side, as stated by CAMPBELL (3) for this species, but are attached in a spiral about the stem. The distribution is irregular; on young stems the leaves are rather crowded and attached along a spiral with about a $\frac{1}{3}$ arrangement. On older stems the distribution approximates a $\frac{3}{5}$ arrangement; fig. 6 shows the arrangement of the leaves on the largest specimen secured. The bases of the leaves inserted on the lower side curve round the rhizome and produce the appearance of a 2-ranked arrangement, as in *Helminthostachys;* but there is nothing in the insertion of the leaves or the structure of the stem to indicate true dorsiventrality.

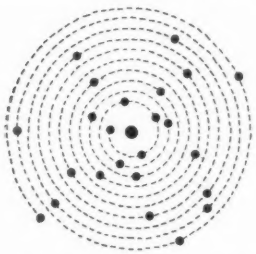

FIG. 6.—Diagram of leaf arrangement of a horizontally growing rhizome.

The rhizomes of the Samoan material are decidedly larger than those from Australia; the oldest one secured, with 8 functional leaves and 7 leaf bases, was 1.2 cm. in diameter and 4.6 cm. in length. Both their appearance and their structure indicate a definite radial arrangement (fig. 5). The leaf insertion is similar to that of the Australian specimens, but the leaves are less crowded.

All the rhizomes in which the bases are intact give evidence of having developed from buds upon roots in the manner described above. The connection of the stem stele with that of the root was examined in 12 specimens; five methods of development of the stem stele were found.

In a single specimen, a solid strand of xylem, surrounded by phloem and endodermis, separates at a considerable angle from one of the protoxylem strands of a tetrarch root (fig. 7). The endo-

dermis soon disappears; before separation from the root stele is complete, a few cells of parenchyma appear near the center of the xylem mass (fig. 7, *C*). The number of these increases and a definite pith is established (fig. 7, *D*). A small gap opens through the xylem, and closes again almost at once; the phloem is not interrupted. The xylem cylinder dilates rapidly and becomes oval in section; root steles attach at the points of the oval, leaving large "root gaps" in the cylinder (fig. 7, *L*). A third root attaches between the two gaps (fig. 7, *M*), producing a small gap; the three gaps close at about the same level. The first strand of the trace

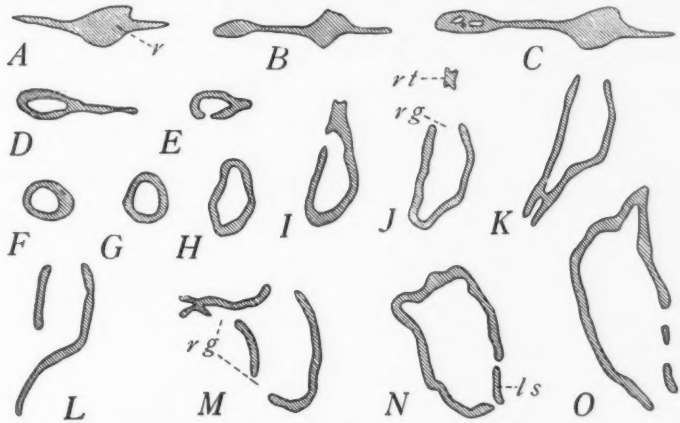

FIG. 7.—Development of stem stele from a single strand; only the xylem is shown: *r*, tetrarch stele of parent root; *rt*, root trace; *rg*, root gap; *ls*, first strand of first leaf; ×12.

of the first leaf separates from the cylinder at a slightly higher level (fig. 7, *N*). This is essentially the course of development of the stem stele of *O. vulgatum* from a bud.

In five cases, two strands of xylem of varying shape in cross-section separate from two of the poles of a triarch or tetrarch root. Diagrams of sections of one of these specimens are given in fig. 8. Each strand at the point of junction with the root stele has phloem on two faces, or surrounding it; the phloem disappears almost immediately from the adjacent faces and becomes restricted to the exterior sides of the strands. After an interval the two strands

fuse at one margin, forming a half-cylinder of xylem (fig. 8, *E*). Two roots are usually given off somewhat farther up (fig. 8, *I*), and the margins of the gap come together at about the level of the base of the first leaf.

In a single instance, three strands arise from the three poles of a triarch root. These fuse just below the point of connection of the first root to form a large strand of semicircular cross-section. The gap between the margins closes just below the level of the first leaf. In another specimen, two strands separate from each of two of the

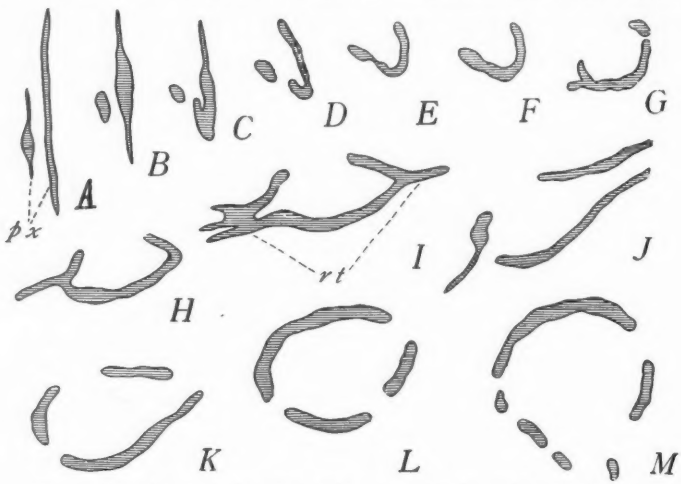

FIG. 8.—Development of stem stele from two strands; only the xylem is shown: *px*, protoxylem strands of parent root; *rt*, root traces; ×12.

protoxylem strands of a tetrarch root. These four organize a stele in the manner described above; the opening in the side is closed rather late, at the level of the top of the first leaf gap.

The stem stele is organized in a distinctly different way in four cases. A series of sections through one of the specimens from below upward is shown by fig. 9. In this case, the xylem mass of one pole of a diarch root begins to enlarge (fig. 9, *A*–*C*). After a time, medullation of the enlarged xylem strand occurs by the appearance of parenchyma near the center (fig. 9, *D*, *E*, fig. 10). The endodermis disappears on the side away from the unchanged

pole of the root. At the same level, gaps appear in the sides of the cylinder (fig. 9, *G*, *H*,) and the xylem separates into two strands. The smaller of these (*r*, fig. 9, *H*) is the continuation of the original root, and at once resumes its original diarch character. The larger strand organizes itself into the stem stele. The three strands of the first leaf separate (fig. 9, *I*, *J*), and at a slightly higher level (fig. 9, *J*, *K*) two roots are attached. The stem stele at this point

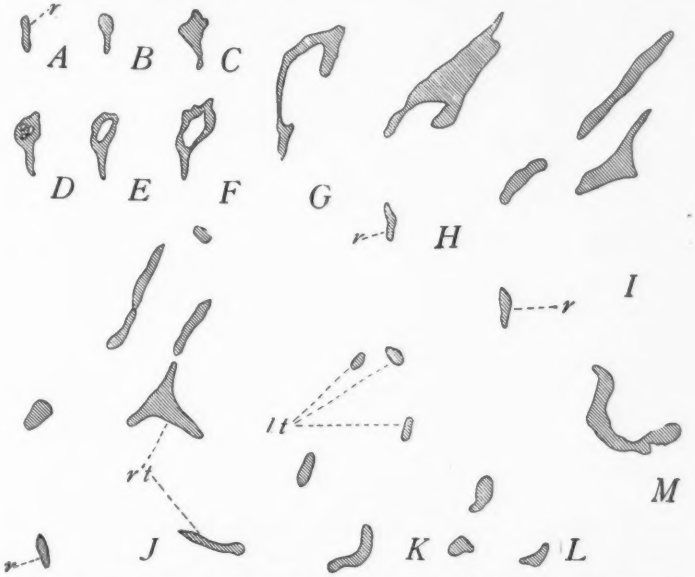

FIG. 9.—Development of stem stele by medullation of root stele; only the xylem is shown: *r*, stele of parent root; *rt*, traces of first roots of new stem; *lt*, trace of first leaf; ×25.

consists of three strands (fig. 9, *L*), which organize the mature form of the stele in the usual way. In the other rhizomes showing this manner of development, the original root is triarch or tetrarch; after separation from the stem stele, the root stele usually shows an increased number of protoxylem strands and the medullated condition shown in fig. 4.

In all cases, the stem stele soon assumes its usual form, that of an ectophloic siphonostele with more or less overlapping leaf gaps

(fig. 11). Two gaps usually occur in the same transverse section, and cause the xylem of the stem to appear as two curved masses with concave sides facing (fig. 11, *G*). Often only a single gap interrupts the cylinder (fig. 11, *A*), or the section may show a complete ring of xylem (fig. 12, *C*); very rarely, three gaps overlap. The leaf gaps are circular or oval and usually very large; 2 mm. in width by 2.5 mm. in height is an average size. The largest observed measures 3.2×3.5 mm. In general the stele is more compact and shows more definitely its cylindrical character than in *O. vulgatum*. It is often very irregular in outline; the insertion of leaf and root strands usually produces a modification of shape, as shown in figs. 11, *I*; 12, *E*; 7, *J*, *K*, etc. It is usually not straight, but slightly bent at each leaf gap in the direction away from the leaf; this is undoubtedly due to the pressure of the young leaf. In diameter the stele is usually a little less than half that of the rhizome, that is, 3–5 mm.

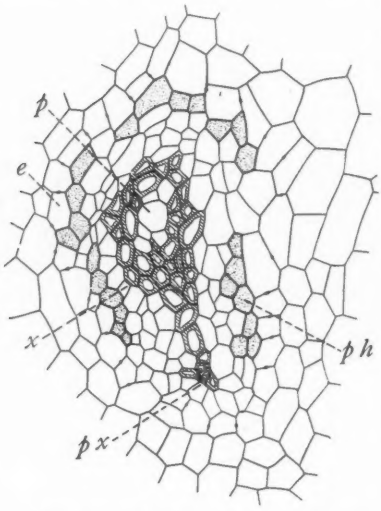

FIG. 10.—Detail of *E*, fig. 9: *x*, xylem; *ph*, phloem; *px*, protoxylem; *p*, parenchyma; *e*, endodermis; ×88.

Besides these leaf gaps, openings definitely related to roots occur in the cylinder (figs. 7, *J*, *L*; 8, *J*, *K*; 11, *B*, *C*, *J*). These are more conspicuous in the larger rhizomes, where a gap occurs above each root; in the smaller specimens, gaps occur in connection with about half of the roots. These openings are narrower in proportion to their length than are the leaf gaps; they range in size from 0.2×0.4 mm. to 0.5×2 mm. Where a root is attached to the cylinder immediately below the point of insertion of a leaf, a gap is almost invariably produced; the leaf strands attach to the sides of this gap, which is therefore continuous with the leaf gap. In cases of this kind,

the gap in the cylinder above the insertion of a root might be interpreted as the beginning of the leaf gap; but in very many cases openings of an exactly similar nature occur when only a root is involved. These gaps close in the same manner as leaf gaps; for convenience they may be referred to as root gaps.

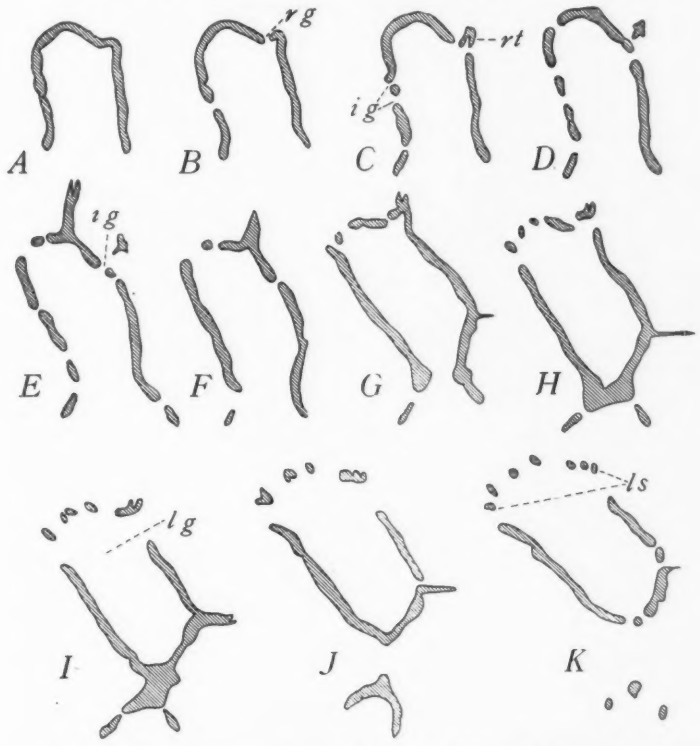

FIG. 11.—Stele of mature rhizome, showing various openings in the cylinder; only the xylem is shown: *rg*, root gap; *rt*, root trace; *ig*, incidental gap; *lg*, leaf gap; *ls*, leaf strands; ×4.5.

In addition to these, openings not related to outgoing strands often occur in the cylinder (fig. 11, *B–F*). These incidental gaps sometimes occur at the margin of a leaf gap; a strand separates from the margin of the gap, runs parallel to it for a time, and later

fuses with it. At other times, a long narrow slit may occur in the cylinder. Some of the gaps are relatively large, measuring 0.6× 0.7 mm. Apparent gaps, due to a failure of the xylem parenchyma to lignify, sometimes occur; in these the phloem is not interrupted. In most of the incidental gaps, however, the cortical parenchyma connects with that of the pith, as in leaf and root gaps.

Lignification occurs first in the layer of tracheids nearest the pith; these first xylem elements differ in no way from those outside. They are true tracheids, often very irregular in shape; lobed and even branched forms occur. They are relatively short, 3–6 times as long as broad; the walls are reticulately thickened. The lignification does not begin at definite points, but indiscriminately throughout the inner layer. It proceeds in an outward direction; in the mature stems the xylem is 5 or 6 tracheids in thickness. Occasional irregular divisions occur within the procambium strand after lignification has begun, but there is no true secondary thickening.

The phloem is uniformly a single layer of cells; it is separated from the xylem by a layer of parenchyma 3–5 cells in thickness. There is no endodermis except in the extreme basal region and at the points of attachment of roots; in these instances it is a mere extension of the root endodermis, and is not to be considered as related to the stem stele. The phloem abuts directly upon the cortical parenchyma composed of large spherical or ellipsoidal cells with intercellular spaces; the cells of the layers next the phloem are somewhat smaller than those farther out. The walls of all the cortical cells are secondarily thickened with cellulose, as in the inner region of the root cortex; the pits are much larger. The pith is in all respects similar to the cortex; the cells of the layers next the xylem are considerably smaller than the average. There is no starch storage in any part of the plant, but fats occur in some quantity in all parts, especially in the pith and cortex of the stem. The growth of the stem is by a tetrahedral apical cell, as in other species of the genus; its segmentation was not examined.

The largest rhizome of the Australian material presents an interesting variation of this usual situation. The base has decayed and it is impossible to say whether the stem originated from a

bud or not. In the lowest part, the cylinder is already definitely organized and of the full diameter. There are five gaps corresponding to leaves that were no longer attached, and 18 leaves were still in position. This is almost certainly the oldest specimen secured.

Up to the level of the fourth leaf gap, the stem cylinder agrees in all details with the usual form, as described above; but about midway of the gap, a strand of xylem separates from the margin of the gap, swings over to the center of the opening, broadens, and by connection with the xylem at either side closes the gap. The gap of the sixth leaf is closed by a similar strand. The gaps of the fifth and seventh leaves are closed in a similar manner by strands which arise as procambium in the parenchyma of the openings. At the level of the fourth leaf, a strand separates from the inner surface of the cylinder opposite the point of connection of a root. This strand runs in the pith near the middle of the cylinder for a distance of about 4.5 mm. and finally closes the gap of the eighth leaf in the manner described. In addition to these five strands, there are in this portion of the stem seven others which arise as procambium or by separation from the inner surface of the cylinder and disappear in the same way, without being in any way related to leaf gaps. One of these closes an incidental gap of the cylinder.

From the level of the eighth leaf to that of the twelfth, there are no medullary strands, and the stele presents the usual appearance. At this point another system of seven strands appears; their positions and behavior are shown in fig. 12. Between the seventeenth and twentieth leaves, no medullary strands occur; but at the level of the latter, three more strands appear as procambium in the pith. One appears in the opening caused by the twenty-first leaf and closes that gap; another appears in the center of the base of the twenty-second leaf, and moves inward and closes the gap. The third, arising near the center of the cylinder, branches once or twice; one branch is recognizable as a procambium strand at a short distance from the apical region.

In all, 23 medullary strands occur: 16 arise as procambium in the pith, 5 as branches from the inner surface of the cylinder, and 2 as branches of other strands; 9 of them are concerned in the

closure of leaf gaps, 7 fuse with the inner surface of the cylinder, 4 disappear by fusion with other strands, and 3 disappear by the gradual fading out of the procambium. They range in length from

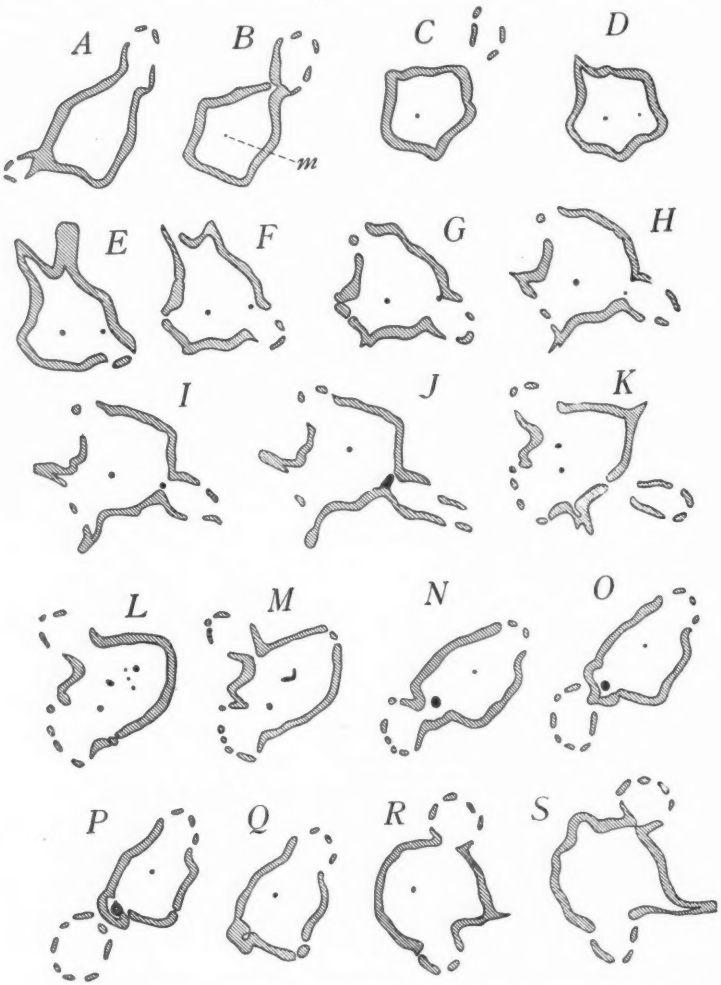

FIG. 12.—Stele with medullary strands; only the xylem is shown: *m*, first medullary strand; ×4.5.

3 or 4 tracheids up to 5.4 mm. in the case of the strand concerned in the closure of the gap of the fifteenth leaf, as shown in fig. 12. A cross-section of one of the strands is shown in fig. 13; they consist of xylem and parenchyma, without a trace of phloem. The largest show 30–40 tracheids in cross-section; 8–12 tracheids is the usual size. No protoxylem can be identified; the tracheids all resemble those of the cylinder.

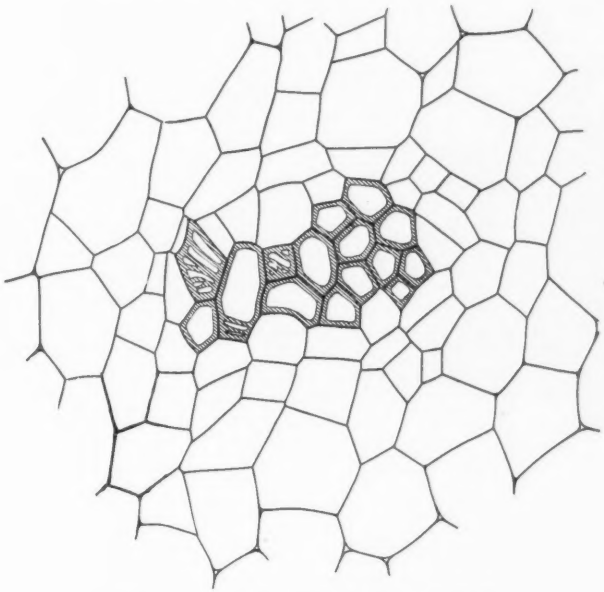

FIG. 13.—Detail of a medullary strand; ×236

In only one other rhizome was anything of this nature observed. This specimen is from Samoa, and has only three leaves; the base is preserved and shows its origin from a bud. At the level of the second leaf a small strand, consisting of a few tracheids only, arises as a procambium at a short distance from the inner surface of the cylinder. It very soon fuses with the cylinder but remains evident as a ridge for a considerable interval. Fron the point of its appearance to that of disappearance is about 0.35 mm.

The leaf

The relation between roots and leaves is very variable. Two or more roots usually appear on the buds before the formation of the first leaf. In the mature stele, roots often connect with the cylinder immediately below a leaf gap; but it is equally common to find two roots attached at the sides of the base of a leaf. No definite relationship between the two can be shown.

As already stated, the leaves in exceptional cases reach a length of 2.7 meters. The stout circular or oval petiole may measure 1 cm. or more in diameter and merges insensibly into the blade; the latter in unbranched leaves is 2–3 cm. in width, but in branched or lobed leaves may reach 5 or 6 cm. at the point of division. Branching and lobing of the leaf occurs very commonly in the Samoan material; in all cases the separation occurs beyond the point of attachment of the fertile spike. There is no abscisss layer, as described for *Botrychium virginianum* by JEFFREY (4).

FIG. 14.—Rhizome with young leaf: *s*, fertile spike; ×1.

In the bud, the tip of the leaf is curved over; the fertile spike when first recognizable is attached just beyond the curve, with its tip directed toward the stem. The original development of the leaf is by an apical cell, but its later enlargement is by intercalary growth. As indicated by fig. 14, this begins at the base and proceeds toward the tip. The portion between the spike and the stem may be 4–5 cm. in length when the portion beyond the spike is only 2–3 mm. long. In the mature leaf, the spike is attached at a point about one-third of the way from the base to the tip of the leaf.

The number of strands separating from the cylinder to constitute the leaf trace varies from 3 to 12; 5 is the commonest number. There is a general relation between the size of the leaf, the size of the gap, and the number of strands. The strands usually attach to the cylinder in a circle, the uppermost one after

the leaf gap is actually closed (fig. 12, *P;* fig. 11, *I*); in some cases, however, all the strands connect within the lower portion of the opening in the cylinder.

The general course of the strands in the leaf base is at a slight angle upward from the point of connection with the cylinder; in very large leaves, the lower ones may run horizontally or even in a downward direction. Branching of some of the strands usually occurs within the cortex of the stem, so that as many as 20 strands may be found in the base of the petiole. This may not occur in small sterile leaves, so that a section of the petiole may show as few as 3 strands.

In the sterile leaves, the strands, 3–5 in number, arrange themselves in the shape of a C, with the opening directed adaxially. The strands in the extremities of the arc in passing up through the petiole swing out toward the margins as the blade is formed, and all come to lie in a single plane with xylem adaxially directed. Frequent branching and anastomosing of the strands occur, so that the number may reach 15 or 20 in the blade of the leaf.

In the fertile leaves, the strands, 4–12 in number, are arranged in a circle or oval as they leave the cylinder, and they maintain this arrangement through the petiole. They branch and anastomose freely, forming a complete cylindrical network; no strands connect across the circle. As the petiole broadens and flattens, the strands arrange themselves in two series: the outer, consisting of 10–15 strands with xylem directed adaxially, form the vascular system of the blade; the inner series, of 5–8 strands with xylem abaxially directed, is reduced by fusions to 4–6 strands which form the vascular supply of the spike. Anastomosing and branching occur as before within each series, but there is no further connection between the two systems. Beyond the point of connection of the fertile spike, the single series of strands constituting the reticulate veining of the blade may increase to as many as 30 in branching leaves. They form a closed system with the exception of a very few small branches which end blindly in the tip.

In the leaf strands as they separate from the cylinder, the xylem elements are all alike, but in the base of the petiole and throughout the leaf the first formed elements can be distinguished by their

smaller size. These protoxylem elements are spiral vessels; they always occur at the inner margin of the xylem, that is, in the endarch position. The protophloem, consisting of 3 or 4 cells, develops at the opposite limit of the strand; the later developed phloem of 6–10 cells is arranged in 2 or 3 layers. One or two layers of parenchyma separate the xylem and phloem in a mature bundle (fig. 15).

In the mature strands of the blade, and, to a less extent, of the petiole, a definite bundle sheath is developed by the thickening of the walls of 2 or 3 layers of parenchyma surrounding the vascular elements (fig. 15). The thickening material is cellulose and the walls are pitted as in similar tissues of the stem and root. This bundle sheath is separated from the protoxylem by 2 or 3 cells of parenchyma, but borders directly upon the protophloem. As a consequence of growth within the bundle sheath, the protophloem in a mature strand is crushed against and between the cells of the sheath.

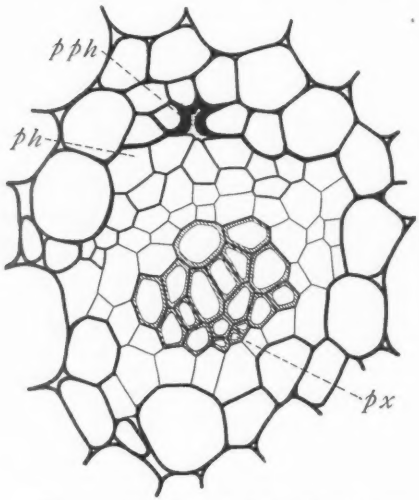

FIG. 15.—Detail of a leaf strand: *pph*, protophloem; *ph*, metaphloem; *px*, protoxylem; ×236.

The spike

As stated above, the strands of the leaf with xylem directed abaxially form the vascular supply of the spike. At the base of the peduncle these are 4–6 in number; they continue with occasional anastomosing and fusion through the median portion of the spike. At the base of the fertile portion of the spike, the strands at the margin of the median region run immediately at the base

of the sporangia. Farther up the spike, where the ridge of sterile tissue which extends into the spore mass is well developed, the strand occupies a position well within this ridge (fig. 16, *B*). There is no branching of the marginal strand below the first few sporangia; but between the third and fourth sporangia, a short lateral branch extending halfway to the edge of the spike usually occurs. Similar strands occur between all the sporangia above this point (fig. 16, *A*). They consist in cross-section of 10 or 12 tracheids, and occupy the center of the thin wall separating adjacent sporangia. Near the margin of the spike they spread out in the shape of a fan and end blindly (fig. 16). The central strands are reduced by fusion to a single strand when the tip of the spike is reached. Between the terminal sporangia this splits into two strands which run out above the sporangia and end in the fan-shaped arrangement described above.

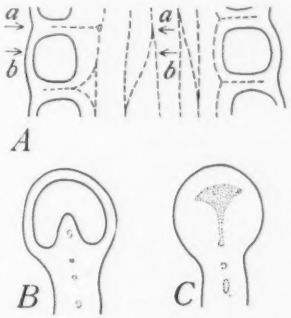

FIG. 16.—Diagrams of vascular system of the spike: *A*, median longitudinal section; *B*, transverse section in plane *bb*; *C*, transverse section in plane *aa*; ×4.5.

The strands of the peduncle and of the median region of the spike show the same structure as those of the blade of the leaf, but the bundle sheath is much less developed. In the strands between the sporangia, the bundle sheath is absent. The protophloem is on the adaxial side of the xylem, but the later developed phloem elements may extend in the shape of a U about the xylem, which is always endarch. In the fan-shaped portion of the strands the phloem cannot be distinguished; there is no distinction between protoxylem and metaxylem.

Discussion

The most striking characteristic of the anatomy of this species is the extreme variability of certain structures. The number of protoxylem strands of the root and the number of strands constituting the leaf trace vary almost directly with the size of the organs concerned. The external conditions which determine whether the

stems are large in diameter or small determine in the same way the number of the strands of the leaf. Such variations may be considered as produced directly by growth conditions and therefore of physiological interest only.

The variability in the connection of the stem stele with that of the root is related to the position in which the bud develops; thus, the bud which developed the stem stele shown in fig. 7 was located directly over one of the protoxylem strands of the root, while that of the specimen shown in fig. 8 was placed midway between two such strands. In the same way, the condition represented in fig. 9 may be ascribed to a more gradual divergence of the root and stem axes; for a time, the two apical cells formed a common tissue within which a single stele was developed, as in fig. 10. When the angle between the two axes became greater, separate steles were developed.

It is of course impossible to say what determines these relations of position of the protoxylem strands and the stem meristem, or the rate of divergence of the two axes; but it seems evident from these variations that the manner of development of the stem stele in a bud is controlled by chance or external conditions. Hence we may conclude that the stelar characters shown in the development from buds cannot be used in any discussion of phylogeny. It is to be noted, however, that two features are constant; these are the collateral arrangement of the stelar elements and the endarch position of the protoxylem.

The occurrence of occasional strands of xylem in the pith is a feature that is unique, so far as the writer is aware. The manner of origin of these strands and their behavior suggests somewhat the medullary strands of *Marattia;* but the absence of phloem distinguishes sharply between the two cases. In any consideration of this feature it should be borne in mind that these strands occur to any considerable extent in only a single specimen.

LANG (**5**) from a study of *Botrychium Lunaria* has concluded that the pith of Ophioglossaceae is purely intrastelar in origin. This opinion is based in part upon the occurrence of scattered tracheids in the pith, particularly in injured rhizomes; this formation of xylem elements from parenchymatous cells of the pith is considered strong evidence of the stelar origin of the pith. The medul-

lary strands of *O. pendulum* afford a much more definite case of this sort; they are in no way a traumatic response; cells of the pith develop a procambium which develops into xylem only; this may be taken to indicate that all cells of the pith are potentially xylem-producing, and therefore stelar.

Summary

1. The root stele varies from diarch to hexarch. The roots branch monopodially, and the branches are diarch at the base.

2. Buds develop upon the roots in the same manner as in *O. vulgatum*.

3. The rhizomes are always radial in structure; the leaves are inserted in an irregular spiral.

4. The connection of the stem stele with that of the parent root is very variable; no phylogenetic significance can be attached to the details of development of the stem stele of a bud.

5. The stele of a mature rhizome is an ectophloic siphonostele with large overlapping leaf gaps; there is no secondary thickening. Root gaps usually occur above the points of insertion of root strands. Incidental gaps not related to outgoing strands occur commonly.

6. In a single large rhizome, numerous xylem strands occur within the pith. These arise from the inner surface of the cylinder or as procambium; some of them are concerned in the closure of leaf gaps, and others disappear as procambium or by fusion with the cylinder. They consist of xylem only.

7. The vascular supply of the leaf consists of 3–12 strands, the number varying with the size of the leaf base. These strands form a cylindrical network in the petiole; in the lower portion of the blade, they constitute two series of strands with xylem oppositely directed. The strands with xylem abaxially directed form the vascular supply of the spike.

The writer is indebted to Professor JOHN M. COULTER for many suggestions and criticisms; and to Dr. CHARLES J. CHAMBERLAIN and Dr. W. J. G. LAND for the material used and for direction during the investigation.

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SOME EFFECTS OF COLLOIDAL METALS ON SPIROGYRA[1]

W. D. HOYT

(WITH FOUR FIGURES)

The effects of colloidal metals on the activities of living cells seem to have received but little attention, although it appears that studies of such effects should yield results of importance to protoplasmic physiology. We regard protoplasm as largely composed of colloidal material, and believe the enzymes affecting many of the protoplasmic processes to be colloidal in their nature. Further, we know that, outside of protoplasm, colloids affect one another in many different ways, and that colloidal metals are capable of bringing about reactions which are similar, in many respects, to those usually related to the action of enzymes. It becomes a matter of considerable interest, therefore, to determine the effects of colloidal metals on protoplasm and protoplasmic products, especially since the soluble salts of many metals are known to be extremely poisonous. The phase of the problem thus suggested with which the present paper has to deal may be stated as follows: Solutions of the salts of silver, gold, and platinum are poisonous to protoplasm; colloidal solutions of the metals named may bring about reactions in non-living materials similar to those caused by enzymes; what, then, may be the effects of such colloidal solutions on protoplasm?

The present investigation was planned to obtain some preliminary information bearing upon the answer to this question. It has mainly to do with the descriptive aspect of some of the superficial effects of colloidal solutions of platinum, gold, and silver on two species of *Spirogyra*, with a few notes on other algae. The work was undertaken at the suggestion of Professor GEORG KLEBS, and was carried out in the Botanical Institute of the University of

[1] Botanical contribution from the Johns Hopkins University, no. 30. These investigations were carried on while the author held the Adam T. Bruce Fellowship of the Johns Hopkins University. A summary of the results was presented before the Botanical Society of America at its Washington meeting, December 28, 1911.

Heidelberg. Unless otherwise stated, the species used was *Spirogyra longata* (Vauch.) Kg. The material and general methods were the same as those described in a previous paper (**8**). The original culture of *Spirogyra longata* was brought from Algiers, but the alga had been growing for nearly three years in the laboratory, in a stock culture which received small additions of tap water from time to time. The filaments of this stock culture were apparently healthy and of the usual appearance. The colloidal solutions of silver, gold, and platinum here employed were made with non-toxic water, according to the method previously described, of distillation in glass with animal charcoal present in the retort. They were kindly prepared for the author by Dr. W. FRAENKEL in the laboratory of Professor G. BREDIG at the University of Heidelberg, by atomizing the metals with an electric arc under water. This method has been described by BREDIG (**2, 3, 4**). The solutions were kept in flasks of Jena glass. Those of gold and platinum were determined by Dr. FRAENKEL and found to contain 90 ppm. (parts per million) of gold and 96 ppm. of platinum, respectively. The sample of the silver solution was unfortunately lost before being determined, but, as all three solutions were originally prepared so as to contain the same amounts of metal (with an error not greater than 10 ppm.), the concentration of the silver solution here employed may be considered as approximately 90 ppm. The solution of gold was purple in color, that of platinum was yellow-brown, while the solution of silver was grayish brown. The gold solution, on standing, formed a deposit, probably of the larger particles, on the walls both of the flask containing the stock solution and of the culture dishes, but the intensity of its color was not perceptibly diminished by this deposition. The solutions of silver and platinum gave no observable deposits. In considering the results obtained with these solutions, the possibility is not to be forgotten that small amounts of metal oxides may have been formed in the process of preparation, and that the solutions are not to be considered as necessarily and entirely free from metal in the ionic condition.

Colloidal solutions of silver, gold, and platinum prepared by BREDIG, similar to those used by the author except that they were weaker and were not made with carbon-distilled water, were

examined by ZSIGMONDY (**17**) with the ultra-microscopic apparatus devised by him. From his results it was calculated that the diameters of the particles in the silver solution were about 50–77 $\mu\mu$. The gold solution contained particles 20–80 $\mu\mu$ in diameter, the larger ones being separable by filtration. There was a much greater range in the size of the suspended platinum particles, but by far the greater portion of these were very small and had an average diameter of 44 $\mu\mu$. As the solutions employed in the present investigation resembled in appearance those described and examined by ZSIGMONDY, it is probable that the particles in these solutions had a similar degree of magnitude.

The results upon toxicity obtained in the present investigation are shown in tables I–IV, which are self-explanatory. In the following consideration of the main points the Roman numerals in parentheses refer to the tables and the Arabic ones to the experiment numbers as there given. .

The solution of silver was extremely toxic (I, 1), killing the alga within 17 hours in concentrations above 0.045 ppm. (0.05 per cent of the solution originally prepared) and injuring many of the filaments in strengths as low as 0.00225 ppm. (0.0025 per cent of the original solution). In filaments thus killed the cell contents were often dark and disorganized and were more or less contracted.

Various substances were added to the colloidal silver solution to determine the effect of their presence upon its toxicity. The addition of salts to form a 0.5 per cent concentration of Crone's nutrient solution[2] to colloidal solutions of silver in concentrations of 0.045 ppm. or less (I, 2), produced marked improvement, as did also the addition of about 81 ppm. colloidal platinum (I, 3), or of about 0.1 gr. animal charcoal (I, 4). Solutions which had previously been injurious or fatal were thus changed into solutions which were entirely or almost entirely non-injurious during the period of the experiments (1–3 days). In such cases the cell contents retained their usual healthy appearance.

The effect of the gold solution shows that it was much less toxic than that of silver. In order to obtain colloidal gold and hold it in

[2] This solution contained salts in the following proportions: 1 gr. KNO_3; 0.5 gr. $MgSO_4$; 0.5 gr. $CaSO_4$; 0.25 gr. $Ca_3(PO_4)_2$; 0.25 gr. $Fe_3(PO_4)_2$.

solution, about 0.02 per cent of sodium hydroxide was added to the water with which the colloidal solution was prepared, so that this solution contained NaOH. Two experiments were carried out with

TABLE I

CULTURES OF SPIROGYRA LONGATA IN COLLOIDAL SILVER SOLUTION, WITH AND WITHOUT ADDITIONS

Culture no.	Silver in medium, approximately, ppm.	Other substances in medium, approximate amounts	Condition of plants*	Duration of experiment, days*
1*a*	90.0	.....	D, D	1, 1
1*b*	45.0	.....	D	1
1*c*	22.5	.....	D	1
1*d*	4.5	.....	D	1
1*e*	0.9	.....	D	1
1*f*	0.108	.....	D	1
1*g*	0.045	.....	P, D	1, 1
1*h*	0.0135	.....	E	1
1*i*	0.009	.....	Gp	3
1*j*	0.0045	.....	E	1
1*k*	0.00225	.....	G, D	3, 1
2*a*	0.045	Salts to form 0.5 p.c. Crone's solution	E, Eg	2, 1
2*b*	0.0135	*do.*	E	1
2*c*	0.009	*do.*	Eg	3
2*d*	0.0045	*do.*	E	1
3*a*	0.045	Colloid. Pt, 81 ppm.	E, E	2, 1
3*b*	0.0135	*do.*	E	1
3*c*	0.009	*do.*	E	3
3*d*	0.0045	*do.*	E	1
4*a*	0.045	Animal charcoal, 0.1 gr.	E, G	2, 1
4*b*	0.0135	*do.*	E	1
4*c*	0.009	*do.*	E	3
4*d*	0.0045	*do.*	E	1

* In tables I–IV the condition of the plants is indicated as follows: *E, excellent,* meaning that none of the filaments seemed injured; *G, good,* meaning that less than half seemed injured; *P, poor,* meaning that more than half seemed injured; *D, dead,* meaning that all or practically all the filaments were dead. A combination of letters denotes a condition between those denoted by the combined letters, respectively; thus, *Eg* means a condition between *excellent* and *good,* etc. Where the experiment was repeated, each of the several notations given refers to a single experiment. The numbers in the last column (duration in days) refer to the experiments in the same order as do the letters; thus, the first number denoting *duration* and the first letter denoting *condition* both refer to the same experiment, etc.

Spirogyra from the stock culture, filaments being transferred to the undiluted, alkaline solution of colloidal gold (II, 5*a*). In one case the filaments remained in excellent condition during the period of the experiment (2 days); in the other case, the alga remained with-

out apparent injury for 5 days, except that the chloroplasts dwindled slightly; but on the sixth day, when the experiment was discontinued, many filaments were dead. For comparison with the above, two experiments were performed with *Spirogyra* which had grown

TABLE II

CULTURES OF SPIROGYRA LONGATA IN SODIUM HYDRATE SOLUTION, WITH AND WITHOUT COLLOIDAL GOLD AND OTHER ADDITIONS

Culture no.	Concentration of NaOH, per cent*	Other substances in medium, approximate amounts, ppm.	Condition of plants	Duration of experiment, days
5*a*†	0.02	Colloid. Au, 90.0	E, Eg	2, 6
5*b*	0.02 [Plants from 0.1 p.c. Crone's sol., 72 days old]	Colloid. Au, 90.0	Eg, P	3, 4
5*c*	0.01	Colloid. Au, 45.0	Gp	2
6	0.02		D	1
7	0.01		Pd	5
8	0.01	Colloid. Pt, 48.0	E	5
9*a*	0.02	Colloid. Ag, 0.0135	D	1
9*b*	0.02	Colloid. Ag, 0.009	Pd	1
9*c*	0.02	Colloid. Ag, 0.0045	Gp	1
10	0.02		Eg, D	1, 1
10*a*	0.01		E, Eg	1, 1
11*a*	0.02	$AuCl_2$, 100.0	P, D	1, 1
11*b*	0.01	$AuCl_2$, 100.0	Eg, Gp	1, 1
12*a*	0.02	Colloid. Pt, 86.4	E, G	1, 1
12*b*	0.01	Colloid. Pt, 86.4	E, E	1,1

*The sodium hydrate solution used in cultures 5*a*–9*c* was a portion of that employed in the preparation of the colloidal gold solution; that used in cultures 10–12*b* was prepared at another time. The strength of the former solution is given only approximately, that of the latter is given accurately.

† Unless otherwise noted, the material of *Spirogyra* used in these experiments was from the stock culture.

in 0.1 per cent Crone's solution for 72 days. The filaments were rinsed in non-toxic water and placed in the undiluted gold solution (II, 5*b*). In one case, the filaments remained in excellent condition two days, but a few showed injury on the third day; in the other case, the alga remained in excellent condition one day, but on the

TABLE III

CULTURES OF SPIROGYRA LONGATA IN COLLOIDAL PLATINUM SOLUTION, WITH AND WITHOUT ADDITIONS

Culture no.	Platinum in medium, approximately, ppm.	Other substances in medium, approximate amounts, per cent	Condition of plants	Duration of experiment, days
13	96.0	.....	Eg, E	22, 3
14*a*	86.4	KCl, 0.1	P	12
14*b*	.....	*do.*, control to 14*a*	Pd	6
15*a*	86.4	$MgSO_4$, 0.02	Gp	19
15*b*	.....	*do.*, control to 15*a*	Pd, D	6, 2
16*a*	48.0	Tap water, 50.0	G	20
16*b*	.....	*do.*, control to 16*a*	Pd	12
16*c*	48.0	Ord. dist. H_2O, 50.0	Gp	26
16*d*	.....	*do.*, control to 16*c*	D, D, D	2, 1, 1
17	.....	$PtCl_4$, 0.008 $MgSO_4$ 0.02	Pd	12

TABLE IV

CULTURES OF SPIROGYRA DECIMINA (NOS. 18–23*b*) AND OF OSCILLATORIA (NO. 24) IN SODIUM HYDRATE SOLUTION, WITH AND WITHOUT ADDITIONS

Culture no.	Concentration of NaOH, per cent*	Other substances in medium, approximate amounts, ppm.	Condition of plants	Duration of experiment, days
18	0.02	Colloid. Au, 90.0	Pd	4
19	0.01	.....	Pd	2
20	0.01	Colloid. Pt, 48.0	E	2
21*a*	0.02	.....	Eg	1
21*b*	0.01	.....	E, Eg	1, 1
22*a*	0.02	$AuCl_2$, 100.0	Eg	1
22*b*	0.01	*do.*	Eg, Gp	1, 1
23*a*	0.02	Colloid. Pt, 86.4	E	1
23*b*	0.01	Colloid. Pt, 86.4	E, E	1, 1
24†	0.02	Colloid. Au, 90.0	E, E	4, 3

* The sodium hydrate solution used in cultures 18–20 and 24 was a portion of that employed in the preparation of the colloidal gold solution; that used in cultures 21*a*–23*b* was prepared at another time.

† The alga used in no. 24 was *Oscillatoria* from a 0.1 per cent Sachs's solution, thickened with 1 per cent agar.

second and third days many filaments were injured, while on the fourth day practically all were dead.

Diluting the gold solution with an equal volume of non-toxic water produced no improvement. In such a solution *Spirogyra* from the stock culture remained in excellent condition one day, but on the second day about half of the filaments were dead (II, 5*c*).

The effect of transferring portions of *Spirogyra* from colloidal gold solution to non-toxic distilled water was tested. Filaments which had been in gold solution for two days, when placed in this water, showed injury within one day and were, for the most part, dead within two days, while the portions of the alga remaining in the gold solution were still in excellent condition. Other effects of such transfers will be described later.

Although the colloidal gold solution itself was only slightly injurious, the sodium hydrate solution (about 0.02 per cent) used in its preparation was extremely toxic, killing all the filaments and causing contraction of their cell contents within 17 hours (II, 6). This alkaline water, when diluted with an equal volume of non-toxic distilled water, was still decidedly toxic (II, 7), but was not injurious during the period of the experiment (5 days) when diluted with an equal volume of the full strength colloidal platinum solution (II, 8). A similar improvement by the addition of colloidal platinum was produced on 0.02 per cent and 0.01 per cent solutions of NaOH prepared at another time (II, 10, 12). Even weak solutions of silver seemed to produce a beneficial effect on this alkaline solution, since the alga lived better with the addition of 0.01–0.005 per cent of the full strength silver solution (0.009–0.0045 ppm.) than in the NaOH solution alone (II, 9). The addition, however, of 0.01 per cent of $AuCl_3$ to 0.02 per cent and 0.01 per cent solutions of NaOH produced no improvement in these solutions; on the contrary, the soluble gold salt seemed to make the NaOH solutions more injurious (II, 10, 11).

The colloidal solution of platinum was still less injurious than that of gold, filaments of *Spirogyra* placed in this solution remaining in apparently perfect condition for 9 days and dying only after 22 days (III, 13). Not only was this platinum solution not injurious during the first few days of the experiments, but it produced

improvement in a 0.1 per cent solution of KCl (III, 14), in a 0.02 per cent solution of $MgSO_4$ (III, 15), and in a mixture with an equal volume of tap water or an equal volume of ordinary distilled water (III, 16), over such solutions without the colloidal platinum. It also, as has been shown above, produced improvement in a weak colloidal solution of silver and in a solution of NaOH. The addition, however, of 0.008 per cent $PtCl_4$ to a 0.02 per cent solution of $MgSO_4$ produced no improvement in this solution (III, 17).

A few experiments were made with other algae. *Spirogyra decimina* Kg.,[3] newly brought to the laboratory, gave practically the same results as those already described, except that it was more quickly injured by colloidal gold solution (IV, 18–23). However, filaments of a species of *Oscillatoria* growing in 0.5 per cent Sachs's solution thickened with 1 per cent agar, when transferred to the gold solution remained in perfect condition and continued their usual movements during the period of the experiment (3–4 days) (IV, 24).

FIG. 1.—*Spirogyra longata* from stock culture, 24 hours after transfer to colloidal gold solution; examined and drawn in gold solution.

When filaments of *Spirogyra longata* from the stock culture or filaments of *S. decimina* were placed in colloidal gold solution, a striking phenomenon was observed; the outer layer of the cell walls swelled irregularly within 17 hours, forming gelatinous sheaths over considerable portions of the external surfaces (fig. 1). These sheaths, whether greatly swollen or not, were colored purple by the gold solution. The cells remained in otherwise excellent condition during this time; their contents were uncontracted and normal in appearance, and the chloroplasts retained their usual green color. That the cells were alive and healthy was indicated by the fact that they were easily plasmolyzed by glycerine. When filaments of *S. longata* were examined directly in the gold solution, they showed slight irregular swellings (fig. 1), but when transferred from the gold solution to glycerine solution or to non-toxic distilled water marked swelling became evident over the entire wall within one or

[3] Determined from O. KIRCHNER, Die mikroskopische Pflanzenwelt des Süsswassers. 1885.

two minutes (figs. 2, 3). Under the latter conditions the modified outer layer frequently became ruptured, breaking away from the wall in purple, gelatinous masses and leaving the remainder of the wall and the other portions of the cell uncolored and apparently unaffected. Filaments of *S. decimina* formed swollen sheaths in the gold solution itself similar to those formed by *S. longata* when transferred from this solution to water. After a

FIG. 2

FIG. 3

FIGS. 2 and 3.—*Spirogyra longata* from same material as that shown in fig. 1, 48 hours after transfer to colloidal gold solution; examined and drawn in distilled water.

sheath had broken from a portion of the wall, no new sheath was formed, the rest of the wall remaining unswollen and uncolored during the period of the experiments (2–6 days). The results of further studies upon the formation of these gelatinous sheaths are presented in table V. Where difficulty was experienced in determining by simple microscopic observation whether or not swelling of the outer wall had occurred, such difficulty was removed by treatment of the material with Bismarck brown, which so stains the unswollen and swollen portions of the walls as to render even very thin sheath layers clearly discernible.

FIG. 4.—*Spirogyra longata* from culture (72 days old) in 0.1 per cent Crone's solution, 48 hours after transfer to colloidal gold solution; examined and drawn in distilled water.

Sheaths were formed in the gold solution by *Spirogyra longata* from the stock culture (V, 1*a*), and by *S. decimina* (V, 1*c*), and they were produced to a very slight degree by a species of *Oscillatoria* (V, 1*d*). No sheaths were thus formed upon filaments of *S. longata* (fig. 4) which had been for 72 days in 0.1 per cent Crone's solution (V, 1*b*), nor by a species of *Hormidium* (V, 1*e*). Sheaths failed to appear upon *S. longata* in colloidal gold solution diluted with an equal volume of non-toxic water (V, 2), nor were they evident upon

TABLE V

VARIOUS TREATMENTS PRODUCING EXTERNAL SHEATHS ON ALGA CELLS, TOGETHER WITH COMPARATIVE TREATMENTS FAILING TO PRODUCE SUCH SHEATHS

CULTURE NO.	ALGA EMPLOYED	MEDIUM EMPLOYED, NON-TOXIC DISTILLED WATER CONTAINING		NUMBER OF TESTS IN WHICH SHEATHS WERE		NOTES*
		Suspensoids and their approximate concentration, ppm.	Solutes and their approximate concentration, p.c.	Formed	Not formed	
1a	*Spirogyra longata*†	Gold, 90.0	NaOH, 0.02‡	2	.....	E; sheaths formed within 17 hours
1b	*Do.*, from culture in 0.1 p.c. Crone's sol. 72 days old	*do.*	*do.*	.....	2	G
1c	*Spirogyra decimina*§	*do.*	*do.*	1	.....	D; within 4 days. Sheaths formed within 17 hours
1d	*Oscillatoria*‖	*do.*	*do.*	1 (?)	1	E; a few filaments in one culture showed slight sheaths when transferred to tap water; no sheaths apparent in gold solution. Usual movements observed
1e	*Hormidium*	*do.*	*do.*	.....	1	.....
2	*Spirogyra longata*	Gold, 45.0	NaOH, 0.01‡	.....	1	.....
3a	*Do.*	.....	$AuCl_2$, 0.01; NaOH, 0.02¶	.....	2	D; many filaments
3b	*S. decimina*	.....	*do.*	.....	1	G
4a	*S. longata*	.....	$AuCl_2$, 0.01; NaOH, 0.01¶	.....	2	G
4b	*S. decimina*	.....	*do.*	.....	2	G
5	*S. longata*	.....	NaOH, 0.02‡	.....	1	D
6a	*Do.*	.....	NaOH, 0.01‡	.....	1	D
6b	*S. decimina*	.....	*do.*	1	.....	No sheaths after 17 hours, but some colorless ones appeared on second day, when filaments mostly D
7a	*S. longata*	.....	NaOH, 0.02¶	.....	2	G, in one culture D, in other
7b	*S. decimina*	.....	*do.*	.....	1	D
8a	*S. longata*	.....	NaOH, 0.01¶	.....	2	G

TABLE V—*Continued*

Culture no.	Alga employed	Medium employed, non-toxic distilled water containing		Number of tests in which sheaths were		Notes*
		Suspensoids and their approximate concentration, ppm.	Solutes and their approximate concentration, p.c.	Formed	Not formed	
8*b*	*S. decimina*	.....	NaOH, 0.01¶	.....	2	G
9	*S. longata*	Platinum, 96.0	.....	.....	1	E, for 9 days D, after 22 days
10	*Do.*	Platinum, 48.0	Tap water, 50.0	.....	1	G
11*a*	*Do.*	*do.*	NaOH, 0.01‡	.....	1	E
11*b*	*S. decimina*	*do.*	*do.*	1	.....	E; heavy sheaths within 17 hours, colored brown by Pt.
12*a*	*S. longata*	Platinum, 86.4	NaOH, 0.02¶	1	1	G; few slight sheaths in one culture
12*b*	*S. decimina*	*do.*	*do.*	1	.....	E; heavy sheaths within 17 hours; colored by Pt.
13*a*	*S. longata*	*do.*	NaOH, 0.01¶	.....	2	E
13*b*	*S. decimina*	*do.*	*do.*	2	.....	E; heavy sheaths within 17 hours, colored by Pt.
14	*S. longata*	Silver, 90, and various lower concentrations	.....	.....	19	Mostly D, in some cases G
15*a*	*Do.*	Silver, 0.135	NaOH, 0.02‡	.....	1	D
15*b*	*Do.*	Silver, 0.09	*do.*	.....	1	Mostly D
15*c*	*Do.*	Silver, 0.045	*do.*	.....	1	Gp
16	*Do.*	Platinum, 96.0; silver, various concentrations below 90.0	.....	.....	5	E
17	*Do.*	Silver, various concentrations below 90.0	Salts to form Crone's solution, 0.5	.....	5	E

* The general condition of the filaments is here denoted by the symbols E, G, etc., as in previous tables.
† All *Spirogyra longata* used was from the stock culture unless otherwise noted.
‡ Sodium hydrate solution employed in the preparation of the colloidal gold solution.
§ The *Spirogyra decimina* was newly brought from the open.
|| The *Oscillatoria* was used from a culture in a 0.5 per cent Sachs's solution thickened with 1 per cent agar.
¶ Sodium hydrate solution as used in preparation of colloidal gold solution, but prepared at another time.

either species of *Spirogyra* in a 0.01 per cent solution of $AuCl_2$ which contained also 0.02 per cent or 0.01 per cent NaOH (V, 3, 4). When filaments of *S. longata* were subjected to the action of the sodium hydrate of the gold solution (approximately 0.02 per cent concentration of NaOH), in the absence of the colloidal gold, no swellings were developed (V, 5). Diluting the sodium hydrate solution by adding an equal volume of non-toxic water was without effect (V, 6*a*). *S. decimina*, however, produced some colorless sheaths on the second day in this 0.01 per cent solution of NaOH, though the majority of the filaments were by this time dead. No sheaths were evident on the first day (V, 6*b*). In sodium hydrate solutions with concentrations of 0.02 and 0.01 per cent, respectively, prepared at another time and perhaps not exactly similar to the solution in which the colloidal gold was formed, neither species of the alga exhibited any swellings (V, 7, 8).

With colloidal platinum no sheaths were formed by *Spirogyra longata* in the undiluted solution (V, 9), nor were they evident in this solution diluted with an equal volume of tap water (V, 10) or with an equal volume of a 0.02 per cent solution of NaOH (V, 11*a*). In this alkaline platinum solution *S. decimina*, on the contrary, formed heavy sheaths within 7 hours, these being colored a deep brown by the platinum (V, 11*b*). Similarly *S. longata* gave only slight sheaths on but a few filaments in slightly diluted platinum solution containing 0.02 per cent of NaOH; it failed to produce any sheaths at all in platinum solution to which 0.01 per cent of NaOH had been added (V, 13*a*). *S. decimina* formed heavy brown sheaths in both of the last named solutions (V, 12*b*, 13*b*).

No sheaths appeared upon *Spirogyra longata* in any strength of silver solution here tested, either with (V, 15) or without (V, 14) NaOH. Nor were sheaths formed by this species in any strength of silver to which was added the undiluted platinum solution (V, 16) or inorganic salts to form 0.5 per cent Crone's solution (V, 17). In these solutions the filaments remained in excellent condition throughout the experiments.

When no swelling became apparent, as in the presence of colloidal platinum alone or in that of colloidal platinum and colloidal silver together, the cell walls remained uncolored, and

seemed to be unaffected by the colloids; but when swelling occurred in the outer portions of the cell walls, as in a mixture of gold or platinum with NaOH, the swollen portions became deeply colored. In these cases the appearance of the colored wall was similar to that produced by the action of a soluble stain like Bismarck brown, a feature which suggests specific adsorption of the suspensoid as the cause of this color change. BREDIG (**2**) and ZSIGMONDY (**16**) mention similar instances of the staining of fungi by colloidal gold. In the former case this was not taken into the filaments but was deposited on the external surfaces of the walls. In the latter case the gold continued to be absorbed until the solution became entirely decolorized.

Apparently the only other published work on the effect of colloidal metals on plants other than bacteria is that of GALEOTTI (**6**). This author carefully studied the effect of colloidal solutions of copper on a species of *Spirogyra* and compared with these the effect of $CuSO_4$ solutions computed to contain the same amounts of copper in the ionic condition. GALEOTTI found colloidal copper poisonous at lower concentrations than was ionic copper, although the action of the former was less rapid. The author concluded that ionic copper produced a rapid effect by combining with the protoplasm, while the colloidal metal, at least in lower concentrations, was slowly active as a catalyser, accelerating certain breaking-down processes within the cells. Addition of 0.01 per cent of NaCl to higher concentrations of colloidal copper rendered these somewhat less toxic and almost entirely corrected the toxicity of the colloid in lower concentrations. The toxicity of ionic copper, on the other hand, was not modified in the presence of 0.01 per cent of NaCl. The influence of the chloride in diminishing the toxic effect of colloidal copper was attributed to some change induced in the colloid itself.

Following GALEOTTI, it may be suggested that the diminution in the toxicity of the lower concentrations of colloidal silver, brought about in the present studies by the inorganic salts of Crone's solution, may have been due to some action of the salts upon the colloid. The effect of animal charcoal and of colloidal platinum upon the toxicity of colloidal silver, that of colloidal platinum upon the

toxicity of toxic waters and salt solutions, and that of colloidal platinum, gold, and silver upon the toxicity of NaOH may be considered as probably due to surface phenomena such as adsorption. It appears from the experiments that ionic gold and ionic platinum ($AuCl_3$ and $PtCl_4$) exerted no influence upon the toxicity of NaOH and $MgSO_4$, respectively. The information at hand is not sufficient to warrant any suggestion as to why the transfer of filaments from colloidal gold solution to non-toxic distilled water was so quickly fatal to *Spirogyra*. A possibly similar case, in which the toxicity of a solution was decreased by addition of a colloid, has been reported by HATCHER (**7**), who found that the toxicity of strychnine injected hypodermically into frogs and guinea-pigs was considerably decreased when the alkaloid was administered in a solution of gum acacia. In this connection it needs only to be suggested that the finely divided and otherwise highly absorbent solids which, as has been shown by various authors since the time of Nägeli (NÄGELI **14**, TRUE and OGLEVEE **15**, BREAZEALE **1**, LIVINGSTON *et al.* **11**, LIVINGSTON **12**, JENSEN **9**, HOYT **8**), exert a correcting influence upon many toxic solutions, partake of some of the properties of colloids. One essential feature, in this regard, of all such solids, as well as of both suspensoids and emulsoids which exert such corrective influence, seems to be great extension of surface.

It is interesting to notice that, although colloidal platinum produced improvement in ordinary distilled water, a weak solution of agar, when added to such water, produced no improvement. It has also been pointed out in another paper (**8**) that a 1 per cent agar hydrogel made with this water was found to be only slightly toxic, while a similar 2 per cent agar hydrogel was decidedly injurious. Evidence is not at hand for any attempt to interpret these phenomena critically.

The results obtained furnish no indication as to what physicochemical properties may be ascribed the extreme toxicity of colloidal silver and copper when colloidal gold and platinum are so slightly injurious. These results agree with those obtained by FOÀ and AGGAZZOTTI (**5**), who showed that, although colloidal platinum, gold, and silver were about equally effective in hindering the development of bacteria, colloidal platinum and gold in strengths of

125 ppm. were fatal to only two kinds of the bacteria tested, while finely divided colloidal silver containing 70 ppm. was fatal to all the bacteria used in their investigations. MORSE (13), from some preliminary experiments on several unicellular animals and plants, concluded that colloidal platinum and gutta percha are without appreciable effect on protoplasm. It should be noted that, in the present investigation, alga filaments in colloidal solutions often showed injury suddenly, after they had remained for several days in apparently excellent or good condition.

The swelling of cell walls caused by akaline colloidal gold or platinum, as observed in these studies, seemed to depend upon the presence of both the colloid and the alkali, since the walls seemed unaffected by the colloid alone and were very slightly altered by a solution of NaOH alone, while marked swelling was produced by a mixture of colloid and NaOH. A mixture of $AuCl_2$ and NaOH was without effect in this connection. The failure of colloidal silver to induce swelling may possibly be associated with those properties of this colloid that produced its toxic effect upon the protoplasm. That no sheaths were formed in those alkaline solutions of silver that permitted the alga to live in apparently good condition may be related to the extreme dilution of these silver solutions.

Results strikingly similar to those obtained in the present investigation from the use of colloidal gold and platinum with NaOH were obtained by KLEBS (10) upon precipitating various substances (as calcium phosphate, copper sulphide, iron ferrocyanide, alizarine-lead oxide) within the gelatinous sheaths of several species of *Zygnema* and other algae. Under these conditions there were formed colored, swollen, gelatinous masses, which separated from the cells in a way quite like that described above. This swelling occurred not only on the walls of living cells, but also on the walls of cells killed by ether vapor or weak alcohol, although not on the walls of those killed by corrosive sublimate, lead acetate, or some other substances. Not all precipitates caused swelling, and the appearance of the gelatinous masses, when formed, differed with different precipitates and different species of algae.

Since the formation of these swollen masses was caused only by precipitates composed of small particles, while it was caused by

widely different chemical substances, and furthermore, since substances deposited as crystals within the sheaths were without effect in this respect, KLEBS concluded that the swelling did not depend on the chemical nature of the precipitated substance, but did depend on the size of the particles composing the precipitate. He was led by these and other considerations to the view that the presence of solid particles between the particles of the gelatinous sheath was the mechanical cause of processes which ended in the breaking-off of the jelly along with the precipitate. He described this result as being brought about by two steps: first, the particles which were precipitated within the sheath slipped out, surrounded by portions of the jelly, and gathered at the periphery, where they were cemented together by the jelly into a layer surrounding each filament; second, this layer containing the precipitated particles was raised up in irregular forms and thrown off by the swelling of the jelly. He concluded that the swelling was not due to a reaction of the protoplasm to a stimulus, that substances which affected the protoplasm also affected the structure of the jelly and consequently its capacity for swelling, but that the observed phenomena were not then explainable in their molecular-physical relation.

On grounds of physical chemistry, as has already been suggested, it seems possible to bring such phenomena as those observed by KLEBS and those of the present studies into the same category with many cases of enzymatic catalysis. Disintegration of cell walls in plants, related to enzyme action, is frequently accompanied by such alterations in the material of the walls as to increase the extent of their imbibing power, so that they swell much more than is usual and assume a gelatinous or mucilaginous consistency. That some colloidal metals (as platinum) may exert influences upon organic material, that are quite similar in many respects to the effects of enzymes, is now generally held. Furthermore, the acidity or alkalinity of the medium employed is well known to influence greatly enzymatic catalysis, some enzymes being more effective in an acid and others in an alkaline solution. That enzymes are characteristically colloid and that their activity is closely related to this fact seems also to be established by the work of many biochemists. The possibility is suggested, therefore, that the disintegration effects

above described, produced in alkaline solutions of colloidal metals, may be considered as catalytic phenomena due to the colloids, the latter acting in a manner more or less similar to that in which the organic colloidal catalytes which we term enzymes are known to operate; if catalysis results frequently from enormously extended surfaces, it may not be without the bounds of probability to suppose that alterations in organic material, similar to those produced by the organic enzymes, may be brought about by inorganic colloids such as those dealt with in the present paper. It seems also possible to relate the swollen cell walls observed by KLEBS, in the study above discussed, to some catalytic action which has altered the water-imbibing power of the wall material, rather than directly to the forces which are active in producing absorption and formation of his precipitates. To attempt the extension of this general hypothesis to the details of the various instances would be out of place at the present time.

It is worthy of note that the swellings with which this paper has had to do were most pronounced in *Spirogyra decimina*, less so in *S. longata* from the stock culture, and were not exhibited at all by the latter form from the culture in Crone's solution. That the last mentioned material was the most vigorous, while the stock material of *S. longata* was next in order, and the material of *S. decimina* was apparently least healthy, suggests that the portion of the cell wall taking part in the swelling differed in the three sorts of filaments; susceptibility to the swelling influence exerted by the colloidal metals seems to have been greater as the alga was less vigorous. Whatever may have been its explanation, we have here another example illustrating the fact that similar filaments of the same algal species, but cultivated under different conditions, may be expected to attain different physiological states, thus becoming physiologically quite dissimilar. Such differences are apparently indicated in the present instance by the diversity above noted in the susceptibility of the outer portions of the cell walls in the three kinds of algal material. The existence of different physiological states in individuals of a single species is of course well known to all experimenters. It has been previously shown for *Spirogyra*, by the present writer (**8**), by parallel and exactly similar experiments

carried out with plants from the same original stock but kept for some time under different cultural conditions. In experiments designed to test the effect of special environmental conditions upon organisms it is absolutely necessary to guard, as far as may be possible, against these different physiological states in the material employed. Such a statement is self-evident, yet many results and criticisms found in physiological literature seem to have been brought into existence through failure to take this fundamental condition sufficiently into account.

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Summary

1. Colloidal silver was fatal to filaments of *Spirogyra* in all concentrations above 0.045 ppm. and was injurious in concentrations as low as 0.00225 ppm. The weaker solutions of silver were rendered almost or entirely non-toxic, during the period of the experiments, by addition of colloidal platinum, animal charcoal, or inorganic salts to form a 0.5 per cent Crone's solution.

2. A solution containing 90 ppm. of colloidal gold and approximately 0.02 per cent of NaOH was only very slightly injurious.

3. A solution containing 96 ppm. of colloidal platinum was almost non-injurious during the period of the experiments, and, in less concentrated solutions, partially corrected the toxicity of tap water, ordinary distilled water, and solutions of KCl, $MgSO_4$, and colloidal silver.

4. Colloidal gold, colloidal platinum, and, to a less extent, colloidal silver, in low concentration, all partially prevented injury to the alga filaments by toxic solutions of NaOH. Addition of $AuCl_3$ to a toxic solution of NaOH, or of $PtCl_4$ to a toxic solution of $MgSO_4$ did not render the hydrate solutions less toxic.

5. When *Spirogyra* was placed in a solution containing colloidal platinum or colloidal gold together with NaOH, the outer portions of the cell walls swelled, forming crumpled, gelatinous sheaths

which became deeply stained by the metal. This swelling was especially pronounced when the filaments were transferred from the alkaline colloidal gold or platinum solution to non-toxic distilled water. The swollen masses thus produced often parted from the rest of the wall, leaving the latter uncolored and apparently unaffected.

6. The cell walls were apparently unaffected by colloidal silver, either alone or with NaOH, or with salts to form a 0.5 per cent Crone's solution. They seemed to be unaffected by colloidal platinum alone or by a mixture of this with colloidal silver. Only very slight swelling of the walls occurred in solutions of NaOH alone. Marked swelling occurred only with the solution of colloidal gold or of colloidal platinum in the presence of NaOH. A solution of $AuCl_2$ and NaOH was without effect in this regard.

7. Filaments of *Spirogyra* originally from the same culture, but grown for a time in different media, exhibited different reactions in the solutions of colloidal gold and NaOH, as well as in the other toxic solutions here employed.

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THE FUNCTION OF MANGANESE IN PLANTS

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Historical introduction

Dating from the time of SCHEELE (1), numerous investigators have noted the presence of manganese in plants of various orders. While small amounts only of this element have been found in most plants, in 1860 HILGARD (2) pointed out that the ash of the long-leaf pine from Mississippi contains a relatively high percentage; and in 1878 J. SCHROEDER (3) found in the ash of the Norway spruce (*Picea excelsa*) 35.53 per cent Mn_3O_4, and in the ash of the bark 41.23 per cent. These and other observations, however, received but little attention for many years. Manganese occurs in small amounts in practically all soils, but being an element unessential to growth and normal development, its absorption by plants was considered to be without physiological significance.

The discovery of BERTRAND (4) in 1897 of the occurrence of manganese in the oxidizing enzymes of plants, however, and the subsequent finding that small amounts of manganese salts stimulate the oxygen-carrying power of these catalytic agents, have drawn attention to this question and have led to the view that after all a physiological rôle is probably played by this element. Since the time of these discoveries a large number of experiments, with a wide range of plants, have been made, various compounds of manganese in water and soil cultures being used; and, in general, it has been found that small amounts of manganese bring about stimulation in growth. While small amounts often produce stimulation, wherever more than a very low concentration has been employed, toxicity has resulted.

LOEW (5) and his co-workers in Japan found, furthermore, the toxic concentration in water cultures to be different for different species of plants. A concentration that was stimulating to rice, for instance, proved to be toxic to barley. Likewise, SALOMONE (6) observed in field experiments that the application of such small amounts as 40 kilograms per hectar of manganous sulphate pro-

duced injurious effects on wheat, while other investigators have found stimulation to result from the application of much larger amounts. In a number of instances, however, the application of different amounts of manganese in field culture has produced no apparent effects.

Regarding the specific effects produced by manganese, BERTRAND, as stated above, and a number of other investigators, have shown that, on the one hand, the addition of small amounts of soluble manganese increases the oxygen-carrying power of the oxidases, and on the other, stimulation is produced in the oxidizing power of infusions from plants grown under the influence of manganese compounds. During recent years an increasing importance has been attached to the oxidizing conditions of the soil, and, as has been pointed out in the researches of SULLIVAN and REID (**7**), there appears to be a direct correlation between the growth of plants (fertility of the soil) and the oxidations going on in the soil. The catalytic oxidations in soils have also been found to be proportional, within certain limits, to the percentage of manganese contained therein, and to be susceptible of stimulation by the addition of manganese compounds in much the same way as stimulation in the activity of plant oxidases is produced by the application of manganese salts.

Certain other investigators have also studied the effects on the solubility of plant nutrient constituents of soils produced by the application of manganese compounds. BERNARDINI (**8**), for example, has shown that manganous chlorid causes a mobilization of calcium and magnesium from both soils and mineral silicates to a greater degree than is produced by potassium, sodium, or ammonium chlorides.[1] From these facts the conclusion has been drawn that plant stimulation, resulting from the application of manganese compounds, is probably due to indirect effects on the inert bases of soils. As bearing on this phase of the question, ASO (**9**) also pointed out that while a 41.8 per cent increase in the yield of rice was obtained from the first application of manganous sulphate, similar applications to the same soil the following year produced only a 2.2 per cent increase.

[1] See NOTTIN, Ann. Sc. Agron. Ser 4. pp. 1–12. 1913.

The toxic effect above mentioned usually manifests itself by a dying-back of the growing tips and the yellowing of the leaves. LOEW and SAWA (**5**) hold that the activity of the oxidizing enzymes of plants may become excessive under the influence of sufficiently great amounts of manganese, resulting in the auto-oxidation of the chloroplastids, thus destroying the green pigment. SALOMONE (**10**), on the other hand, found evidences of plasmolysis in wheat that had been fertilized with manganese dioxide at the rate of fifty kilograms per hectar. BRENCHLEY (**11**) also observed a toxic action from manganous sulphate when it was applied in the very low concentration of one part per one hundred thousand parts of culture solutions. When it is remembered, however, that many plants vegetate normally in culture solutions varying rather widely in ionic-concentration, without showing evidences of plasmolysis, in other words, the ionic-concentration of the cell sap considerably exceeds that of ordinary culture solutions, it seems improbable that the addition of such small amounts of manganous sulphate would produce plasmolysis as a direct effect.

From the foregoing brief résumé[2] of the experiments on this question, it will be seen that two different sets of views have been held by which the action of manganese on soils and plants has been explained. These may be briefly stated as follows: (1) manganese stimulates the necessary oxidations going on in soils and plants through the activation of the oxidizing enzymes, etc.; (2) the application of soluble manganese brings about plant stimulation by rendering soluble, and therefore making available, the essential plant food of soils.

Under the first named of these theories may be included the stimulated oxidations, in both soils and plant tissues, the former probably being referable to the action on certain microorganisms, as well as on soil catalysis, while the latter has to do with the physiological processes within the cell. Viewed in the light of the last named theory, the effects of manganese on plants are considered to be indirect and due to its increasing the solubility of the mineral constituents of soils.

[2] A more complete bibliography of literature dealing with this subject will be found in a paper by the writer published as Bulletin 26, Hawaii Experiment Station.

A study of the literature on this subject, embracing as it does experiments with a considerable range of plants, reveals discrepancies, and, in the opinion of the writer, casts doubt on the adequacy of either one or both of the above mentioned conceptions as furnishing sufficient basis for a complete explanation of the function of manganese in plants. In this connection, the application of the principles of plant physiology, particularly osmosis in its bearing on the absorption of chemical substances from soils, naturally raises the question of the influence of soluble manganese on the selective absorption of the necessary nutrient elements. SCHREINER and SKINNER (**12**) have shown, for example, that small amounts of cumarin materially alter the absorption of potash by wheat seedlings; that quinone modifies the osmotic absorption of phosphoric acid; and that vanillin and dihydroxystearic acid exert a noteworthy influence upon the absorption of nitrates. Up to the present time the fundamental steps and the specifics of these reactions have not been elucidated. The exact "how" as yet can only be inferred. With regard to the specific effects on the osmotic phenomenon produced by small amounts of inorganic elements occurring in soils, other than the so-called plant food elements, little indeed is known. It is reasonable to suppose, a priori, that some osmotic effect would be produced. In fact, it seems improbable that the presence of any considerable amount of an electrolyte in the nutrient solution would exert a strictly neutral effect.

For a number of years the writer has studied the effect of manganese on plants. A preliminary report of this work was published in 1908 (**13**), in which it was shown that certain soils of Oahu, used for pineapples, contain abnormally high percentages of manganese. On these areas the pineapple undergoes the phenomenon of chlorosis, the leaves becoming yellow and the fruit pink in color throughout the entire period of its growth. The growth of the plant is stunted and the fruit produced is inferior in size. Further investigation of this question has led to a study of the function of manganese in plants in general, the results of which, in view of the prominence of the question, appear to be of sufficient interest to warrant a brief discussion at this time.

Experimental observations

The macroscopic appearance of a number of plants when grown on manganiferous soil is characteristic. Pineapples develop chlorosis at an early age, from which they never recover. The effects are first noticeable by a yellowing on the margins of the leaves or the development of yellow spots which soon spread over the entire leaf. The growing tips die back, the leaf margins becoming brown. Usually such plants produce small fruit of an abnormally pink color. Pineapple roots, instead of maintaining the usual pointed growing tip, are often found to be greatly enlarged, sometimes to the size of a lead pencil. The roots of other plants also become modified to some extent. Those of *Panicum molle* are found to be abnormally woody. Others are similarly affected. Corn makes poor growth, the leaves turning brown at an early stage and the stalks taking on a deep purple color. Barley, oats, and rice are likewise stunted in growth. Of the Leguminosae, the cowpea (*Vigna catjang*), in particular, is very sensitive to manganese; the lower leaves become brown, die back from the tips, and fall away. The pigeon pea (*Cajanus indicus*) is affected somewhat similarly. Onions also die back from the tips and produce very small bulbs.

Some species, however, appear not to be affected. The *Agave sisalana* shows no effects; sugar cane is not greatly affected, and cotton and tobacco, although somewhat retarded in growth, are not otherwise affected. *Waltheria americana*, the sow thistle, and a species of *Crotalaria* grow as weeds on the manganiferous soils and are in no way hindered in their development. These illustrations could be greatly enlarged, but are sufficient to indicate that the apparent effects of manganese in different species are far from being uniform.

Microscopical studies.—From a microscopical study of the several parts of these plants, it was found that in those instances where manganese exerts a toxic effect, the cell walls of the root cortex become brown, and in some instances contain minute granules of manganese dioxide. In a few instances the cells having brown cell walls extend into the central cylinder of the root, and

it is not uncommon to find a thickened tissue lying next to the cell walls in the angles of the cells. In the more advanced stages of chlorosis the protoplasmic contents of pineapple leaves become contracted into formless masses and appear to undergo partial decomposition. Normally, the palisade cells (**14**) of this species contain not more than mere traces of chlorophyll, being essentially water-storage tissue. Under the influence of manganese the liquid content becomes coagulated and draws away from the cell walls; likewise the protoplasm in the chlorophyll-bearing cells breaks away from the cell walls in places, contracting into an irregularly shaped mass. Plasmolysis, therefore, takes place, and in a few instances the nuclei become brown.

At the beginning of the chlorosis there is a fading of the green color without any other noticeable change; but in successive stages the chloroplastids gradually become fewer in number and smaller in size until finally they almost completely disappear. Simultaneously with the fading of the chlorophyll, a diminution in the starch contents takes place, and as the chlorosis develops, less and less starch is found adhering to the chloroplastids, until finally it entirely disappears. In the chlorotic plants the palisade cells also seem to be given over to the storage of calcium oxalate, and the occurrence of this metabolic by-product is greatly increased in practically all parts of such plants.

OXIDIZING ENZYMES.—The view that manganese exerts an influence on plants, through stimulating the auto-oxidations (so indispensable to living matter), gives interest to a study of the oxidizing enzymes occurring in plants from manganiferous soils. Accordingly, the oxidase and peroxidase activities of infusions obtained by crushing the leaves were measured empirically by use of the guiacum and aloin reactions. The results showed that while infusions from some chlorotic plants contain vigorous oxidizing enzymes, frequently such infusions give much weaker oxidase and peroxidase reactions than normal plants. These tests have been applied to infusions from all parts of pineapple plants, coming from soils containing manganese in amounts varying from 0.01 to 9.75 per cent, and with plants at all ages and in the various stages in the development of chlorosis; but when applied to a large series of

samples no correlation was found between the percentage of manganese in the soil or the degree of chlorosis on the one hand, and the activity of the oxidizing enzymes on the other.

These tests have also been made with various other plants, some of which show toxic effects from the manganese, and with still others that appear not to be affected. On the whole, the results again fail to show any relation between the activity of the oxidizing enzymes and the percentage of manganese in the soil, or the toxic effects.

As is well known, the activity of oxidizing enzymes in different specimens of a given species of plant is by no means uniform when grown on normal soils. It has been found, for instance, that extracts obtained from the fresh leaves of normal sugar cane and pineapples, respectively, varied in their oxygen-carrying power between wide extremes. It is also known that pathological disturbances, caused by attacks of Aphidae, the mosaic disease of tobacco, etc., are also associated with accelerated oxidation. The autumnal yellowing of plants, incident to maturity, and the development of yellow spots on certain plants, have likewise been shown by Woods (**15**) to be associated with an increased activity of the oxidases and peroxidases.

It seems reasonable to suppose, therefore, that while manganese has the power of increasing the oxygen-carrying power of oxidases, and consequently may, in this way, to some degree bring about plant stimulation, the phenomenon of chlorosis cannot be completely explained on the basis of excessive auto-oxidation. The development of chlorosis under the influence of manganese is very probably the result of physiological disturbances of a more deep-seated nature.

Composition of the ash.—A number of ash analyses have been made for the purpose of determining the effects of manganese on the absolute, as well as the relative, absorption of plant food elements. The materials for analysis were selected with the greatest care, so as to secure representative plants of the same age and stage of development in a given species. The results are recorded in table I.

The data given in table I bring out some interesting facts. In

TABLE I

ASH ANALYSIS OF PLANTS GROWN ON NORMAL AND MANGANIFEROUS SOILS

Plants analyzed	Manganese (Mn_3O_4)	Lime (CaO)	Magnesia (MgO)	Phosphoric acid (P_2O_5)	Total ash
Pineapple leaves 5 months old:	*P ct.*	*P ct.*	*P ct.*	*P ct.*	*P ct.*
Manganiferous soil	2.41	9.01	5.70	2.81	9.94
Normal soil	1.70	7.14	7.60	3.57	7.14
Pineapple leaves 18 months old:					
Manganiferous soil	2.08	15.66	7.91	1.66	7.98
Normal soil	1.40	7.00	6.98	2.70	6.24
Pineapple stalk 5 months old:					
Manganiferous soil	.25	36.42	2.60	6.12	8.85
Normal soil	.25	23.87	5.82	8.86	5.12
Pineapple stalk 2 years old:					
Manganiferous soil	.80	14.36	7.75	6.70	7.78
Normal soil	.20	12.96	5.78	8.36	6.60
Corn stover:					
Manganiferous soil	.40	8.60	7.64	5.17	7.55
Normal soil	.15	3.45	4.60	7.56	9.18
Cowpea vines:					
Manganiferous soil	3.07	22.72	6.93	2.90	13.73
Normal soil	.87	17.10	9.13	5.03	13.44
Cowpeas:					
Manganiferous soil	.15	1.47	4.15	15.89	3.93
Normal soil	.00	1.13	6.91	22.71	3.53
Paspalum orbiculare:					
Manganiferous soil	2.20	5.30	3.46	2.54	7.88
Normal soil	1.05	6.70	5.05	2.34	7.16
Guava leaves:					
Manganiferous soil	.82	43.02	3.73	4.47	7.66
Normal soil	.28	22.98	10.46	7.70	6.73
Guava wood:					
Manganiferous soil	1.20	52.10	1.51	4.33	5.87
Normal soil	.15	26.17	7.22	6.97	4.02
Sugar-cane leaves:					
Manganiferous soil	.45	14.33	4.41	3.21	5.57
Normal soil	.60	16.00	6.07	4.03	4.85
Crotalaria:					
Manganiferous soil	1.40	30.80	7.79	4.12	6.77
Normal soil	.05	19.00	21.05	9.13	6.28
Peanut leaves:					
Manganiferous soil	.25	35.24	5.61	4.49	10.45
Normal soil	.04	39.41	10.99	4.97	9.95
Peanut stems:					
Manganiferous soil	2.56	14.80	9.04	2.00	11.15
Normal soil	.32	21.76	19.02	4.81	7.12
Ironwood (*Casuarina equisetifolia*) needles:					
Manganiferous soil	.66	37.50	4.57	1.62	.....
Normal soil	T.	43.12	7.75	4.23	.....
Olive leaves:					
Manganiferous soil	.64	26.32	1.84	2.63	.....
Normal soil	.86	17.80	1.53	2.78	.....
Waltheria americana leaves:					
Manganiferous soil	8.70	31.30	3.81	2.80	.....
Normal soil	.82	29.62	5.44	3.81	.....

TABLE I—*Continued*

Plants analyzed	Manganese (Mn_3O_4)	Lime (CaO)	Magnesia (MgO)	Phosphoric acid (P_2O_5)	Total ash
Waltheria americana stems:					
Manganiferous soil	.56	14.97	11.70	3.67	3.58
Normal soil	.45	13.70	11.30	3.46	3.73
Broom-corn leaves:					
Manganiferous soil	2.24	12.08	6.75	.94	.....
Normal soil	.60	5.44	3.52	1.38	.....
Broom-corn stalks:					
Manganiferous soil	1.36	2.12	3.12	1.02	.....
Normal soil	.52	1.88	2.51	3.72	.....
Tobacco stems:					
Manganiferous soil	.57	9.47	4.08	2.08	7.02
Normal soil	T.	1.88	3.27	7.35	8.50
Pigeon-pea leaves:					
Manganiferous soil	1.43	16.11	3.25	4.04	12.17
Normal soil	.15	7.86	7.66	10.85	7.84
Pigeon-pea stems:					
Manganiferous soil	.99	15.29	2.82	4.25	5.81
Normal soil	T.	3.79	4.39	9.34	6.22
Oat straw:					
Manganiferous soil	.86	9.15	5.16	.73	10.27
Normal soil	T.	3.40	3.19	8.81	11.73
Wheat straw:					
Manganiferous soil	.22	4.51	4.03	2.96	8.91
Normal soil	T.	2.09	2.74	5.56	16.27
Mango leaves:					
Manganiferous soil	2.16	33.12	2.15	2.89	9.24
Normal soil	.24	17.13	4.90	3.43	8.21

practically every instance the absorption of manganese was increased on the manganese soil. The analyses further show that there is a pronounced difference in the percentages of lime, magnesia, and phosphoric acid in the ash of plants from the two classes of soils. Some of the plants were not visibly affected; others little so; while still others showed a pronounced toxic effect; but uniformly throughout there was a tendency toward an increased absorption of lime on the one hand, and a decreased absorption of magnesia and phosphoric acid on the other.

Under certain conditions the ratio of lime to magnesia seems to be of considerable importance to plants. In order to bring out more clearly the relations between these two constituents of the ash, the relative amounts of lime and magnesia absorbed from normal and manganiferous soils, respectively, have been recalculated from the previous ash analyses and are presented in table II.

An inspection of the data in table II shows that in almost every

TABLE II

THE RATIO OF LIME TO MAGNESIA IN PLANTS (MAGNESIA CONSIDERED AS 1)

Kind of plant	From manganiferous soil	From normal soil	Kind of plant	From manganiferous soil	From normal soil
Pineapple leaves:			Ironwood needles	8.20	5.56
5 months old	1.58	.94	Olive leaves	14.30	11.63
18 months old	1.98	1.00	Waltheria americana:		
Pineapple stalk:			Leaves	8.21	5.44
5 months old	14.00	4.10	Stems	1.28	1.21
18 months old	1.88	2.24	Broom corn:		
Corn stover	1.12	.75	Leaves	1.79	1.54
Cowpeas:			Stems	.67	.74
Vines	3.28	1.86	Tobacco stems	2.32	.57
Seed	.35	.16	Pigeon peas:		
Paspalum orbiculare	1.53	1.32	Leaves	4.69	1.02
Guava:			Stems	5.42	.86
Leaves	11.53	2.19	Oat straw	1.79	1.07
Stems	34.50	3.62	Wheat straw	1.12	.76
Sugar cane leaves	3.25	2.73	Mango leaves	15.40	3.49
Crotalaria	3.95	.90			
Peanut:					
Leaves	6.28	3.58			
Stems	1.63	1.14			

instance the ratio of the absorbed lime to absorbed magnesia was increased under the influence of manganese.

Discussion

In this investigation it has been shown that different plants, when grown on manganiferous soils, are affected differently. Some species are stunted in growth and die back from the tips of the leaves, which turn yellow or brown, and sometimes fall off, and a general unhealthy appearance results. Other species appear to be unaffected, and, so far as can be judged, vegetate normally in the presence of manganese. Microscopic investigations show that in certain instances the protoplasm undergoes changes. Occasionally it draws away from the cell walls and the nuclei become brown. There is a manifest change in the protoplasmic contents of the roots.

The chlorophyll in a number of plants is affected; in pineapples it undergoes decomposition. Simultaneous with the destruction of chlorophyll, starch formation ceases.

The activity of the oxidizing enzymes in plants is herein shown to bear no relation to the destruction of chlorophyll under the

influence of excessive manganese. While the oxidases generally contain manganese as a normal constituent, or at least manganese is closely associated with the oxidases, and at the same time their oxygen-carrying power is accelerated by the presence of manganese salts, the foregoing investigations show that there is no correlation between the phenomenon of chlorosis in pineapples and the activity of the oxidizing enzymes. The decomposition of chlorophyll in this case, therefore, is not due to excessive autoxidation. This does not imply, however, that accelerated autoxidation in plants is without effect.

From the ash analyses it was found that manganese was absorbed in considerable quantities, and in nearly every instance was greater in the plants from manganiferous soil. These analyses also show that a disturbance in the mineral balance takes place. The percentage of lime is increased, while the percentage of magnesia and phosphoric acid is decreased. Some of the plants analyzed showed a markedly toxic effect from the manganese, while others appeared to be unaffected; but in practically every instance a modification of the mineral balance was observed, and this was found to follow the same direction in all species. The ratio of absorbed lime to the absorbed magnesia was increased under the influence of manganese, regardless of whether the plant showed a toxic effect or not.

It is claimed by LOEW (**16**) and others that various plants are affected differently by different ratios of lime to magnesia; certain species of plants vegetate most advantageously when the ratio is one to one; whereas still other species grow best when the ratio is one to three, etc. In the publications dealing with this subject, however, mention is usually made of the effects produced upon the appearance of plants, while but few ash analyses have been made in this connection. From the data at hand, however, it appears that where the physiological balance between lime and magnesia is disturbed, a corresponding influence is brought about in the composition of the ash.

Under the influence of manganese, plants automatically modify themselves in regard to the absorption of these two elements, and, as is believed, it is not so much the absolute amount of calcium and

magnesium in a given soil as the ratio between these two substances in solution in the soil moisture that determines the physiological effects. In the case of plants grown on manganiferous soil, the relative amounts of lime and magnesia actually absorbed become greatly different from those absorbed from normal soils, regardless of the amounts of these elements present in the soil.

From these evidences, we may believe, then, that an important effect of manganese is of an indirect nature, being due to its bringing about a modification in the osmotic absorption of lime and magnesia, and that the toxic effects are chiefly brought about by this modification, rather than as a direct effect of the manganese itself. As has been mentioned already, not all species of plants are equally sensitive to modifications in the lime-magnesia ratio; and from some recent experiments of GILE (17) it seems that the effects of a modification in this ratio are, to some extent, dependent upon the concentration of the culture solution. Likewise, different ratios are best suited to different species. Therefore, the effect of manganese may be very different on different soils and with different species of plants. With certain plants it is toxic on certain soils for the reason that the absorbed lime and magnesia are thrown out of their optimum ratio for this plant, while in others it may exert a stimulating effect by bringing this ratio more nearly to its optimum.

The small amounts of manganese in natural soils, therefore, probably perform a twofold function in plant growth: (1) it acts catalytically, increasing the oxidations in the soil and accelerating the auto-oxidations in plants; and (2) it tends to modify the absorption of lime and magnesia, perhaps by partially replacing calcium from insoluble combinations, but especially, through a direct effect on the osmotic absorption of lime and magnesia, increasing the former and decreasing the latter.

The absorption of phosphoric acid is likewise decreased in the presence of manganese. By reference to the preceding table of ash analyses it will be seen that frequently the ash of a given species of plant from manganese soil was found to contain not more than one-half as much phosphoric acid as from normal soil. The interference with the absorption of phosphoric acid would also tend to

bring about stunted growth and might be sufficient to account for the difference in size of the plants from the two classes of soil. Studies on the solubility of these soils have shown the manganese to be slightly soluble in water, but markedly so in dilute organic acids. Phosphoric acid coming into solution in the soil moisture would tend to be precipitated by the manganese as manganese phosphate, a compound, which can be dissolved only with difficulty, and therefore the absorption of phosphoric acid would thus be hindered.

Reference has already been made to the fact that certain organic substances exert appreciable influence on the rates of absorption of certain of the necessary elements. Here it is shown that a similar action is to be ascribed to manganese. How is this fact to be explained?

During recent years the protective action of salts shown to obtain in animal experiments by LOEB (**18**) has been found to apply also to plants.

OSTERHOUT (**19**) has shown from water cultures, for instance, that some of the essential elements may be toxic unless properly balanced by definite concentrations of certain other necessary elements. In other words, some at least of the essential inorganic elements seem to play both a nutritive and a protective rôle. Quite recently OSTERHOUT (**20**) has been able to throw considerable light on the mechanics of the protective action. By the use of an ingenious device he determined the electrical conductivity of living protoplasm, both before and after it was placed in solutions of sodium and calcium chlorides, etc. The results show that the electrical resistance of the protoplasm becomes greatly reduced upon standing in sodium chloride solution, thus indicating that sodium had been absorbed. But when the protoplasm was placed in a solution containing sodium and calcium chlorides, of the same ionic concentration, no such lowering of the resistance took place. Calcium hinders, then, the passage of sodium through living protoplasm; and therefore must be looked upon as lowering the permeability. OSTERHOUT has further shown this phenomenon to be reversible, and therefore the presence of one salt may actually cause an increase in the permeability to others. In certain instances

he found the protoplasm to undergo visible changes, which, however, were not necessarily injurious to it. Protoplasm, being of a colloidal nature, then, becomes physico-chemically altered when placed in certain solutions; and such alterations, in turn, affect its permeability.

By the application of the above named conceptions, we may infer that when plants are grown on manganese soils, the soluble manganese, coming into contact with the root hairs, is at first absorbed, up to a certain point, forming combinations with the protoplasm. Once these combinations are established, the permeability of the protoplasm becomes altered, whereby the absorption of lime is facilitated, while that of magnesium is hindered. Manganese, then, may be looked upon as forming a combination with the protoplasm which materially alters the relative absorption of lime and magnesia. Not all plants are equally affected by a variation from the normal ratio of lime to magnesia, and therefore under field conditions toxic effects may be produced in some plants, stimulation in others, while with still others the effects may be neutral.

The experimental facts presented in this paper and the interpretations made in no way negative the work previously done on this subject. The failure to observe a more active catalytic oxidizing reaction in plants from highly manganiferous soil does not argue that manganese is incapable of stimulating the activity of oxidizing enzymes. The normal soils of Oahu, from which plants were taken for comparison, contain small amounts of manganese, which, moreover, was found to be absorbed to a considerable extent by all the plants studied. It is probable, therefore, that the absorption of manganese from the normal soils of the Islands is sufficient to produce maximum stimulation in oxidase activity.

HAWAII EXPERIMENT STATION
HONOLULU

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THE DEVELOPMENT OF THE PROTHALLIUM OF CAMPTOSORUS RHIZOPHYLLUS

F. L. PICKETT

(WITH PLATES XII AND XIII AND EIGHT TEXT FIGURES)

In this region the spores of *Camptosorus rhizophyllus* mature from June to October. The long axis measures 18–24 μ, and the shorter 12–20 μ. They contain a few small oil globules, but are destitute of chlorophyll. The nucleus is inconspicuous in the living spore. The perinium is thick, dark, and shows many sharp, irregular ridges with areas of unequal thickness intervening (figs. 47–55). The exospore is seen with difficulty, if at all, as the perinium is very rarely pushed off when the spore germinates.

The spores germinate slowly. The greater part of the writer's material was taken from fronds collected October 26, 1912, and kept between sheets of filter paper in a book in the laboratory. On November 22 the sporangia, after being scraped from the fronds, and crushed lightly to free the spores, were sown on well sterilized soil in clay saucers. The saucers of soil were protected from currents of air by bell-jars supported on small blocks of wood to allow proper ventilation, and were kept moist in a well lighted greenhouse at an average temperature a little above 70° F. The first sign of green was noted with a magnifier on December 17. On February 4, 1913, abundant antheridia were found on the larger prothallia, and one week later a few archegonia were found. On March 22 many old antheridia and archegonia could be found on the large prothallia. Other spores collected October 26, 1912, were sown December 23, and kept under the same conditions as the above, but out of direct sunlight. In these cultures the first sign of green was noted January 30, 1913. Other spores sown January 3 and kept in the light, where on days of bright sunshine the temperature reached 80° F. at midday, showed the first sign of green on January 30. In all these instances the prothallia were composed of 3–10 cells before their color was noted on the soil. Spores sown on boiled tap water, and kept under the same conditions

as the above mentioned cultures, showed the beginning of germination in 10–14 days. While spores collected in October germinated best before the first of February, a considerable percentage germinated as late as the last of March, having been kept in the dry air of the laboratory in the meantime. Spores collected from the usual habitat in March show a higher percentage of germination than those collected earlier and kept in the laboratory. Only a few mature; unopened sporangia may be found in the field as late as March, so but few spores can be secured at that time.

All the spores sown at one time and under the same conditions do not germinate and develop uniformly into prothallia. A small bit of soil, bearing prothallia, taken from any one of the cultures mentioned above, would show, up to the first of May, widely different stages of prothallial growth. Many times the writer has found on the same bit of soil the intermediate stages from prothallia composed of two or three cells to mature plants 2–3 mm. wide, and bearing antheridia and archegonia. That some of the small prothallia were dwarf male plants is beyond doubt, because of the presence of antheridia. But that some of the spores were late in germination is clearly indicated by the constant recurrence of these gradations in development accompanied by a continual increase in the number of mature specimens. The plants from which the photographs reproduced in pl. XIII were made were taken at one time from the same portion of one culture, and had exactly the same conditions for germination and growth.

Germination of the spore

When the spores are placed under the proper conditions for germination, they absorb water and increase in diameter from one-fourth to one-half. The swelling is followed by a rupture of the perinium and the exposure of the spore contents. The writer has been unable as yet to determine whether or not there are two distinct walls within the perinium. If present, they are quite transparent. Through the opening in the perinium may be seen the oil globules, sometimes an inconspicuous nucleus, and after a few hours a few small chloroplasts. From this point two lines of development have been noted.

In the first case, as in the typical Leptosporangiatae, a small papilla is formed and cut off from the body cell by a wall. This papilla elongates to form the first rhizoid. It contains no chloroplasts. Occasionally two such papillae are formed before the division of the prothallial cell (figs. 54, 55). Following the formation of the first rhizoid, the body cell increases in size, the oil globules disappear, and the chloroplasts increase in size and number. The second division of the spore produces a new prothallial cell containing chloroplasts.

In the second case, the first division may produce two prothallial cells similar in size, and both containing chloroplasts. The basal cell of such a group may later produce a rhizoid. From the examination of many germinating spores and young prothallia, it seems quite as common for the spore to undergo one or two divisions before the formation of a rhizoid as for the rhizoid to result from the first cell division. Numerous specimens similar to fig. 52, but without a rhizoid, have been seen. As germination proceeds, the perinium is distended but is not cast off; it may usually be found attached to the oldest cell of mature prothallia (figs. 14, 15).

As has been stated, immediately after the rupture of the walls, light green plastids appear in the exposed portion of the cell (figs. 51, 54). With the further growth of the spore and the formation of new cells, these chloroplasts become larger and increase rapidly in number. Throughout the life of the prothallium the chloroplasts retain a distinct yellow tinge, imparting a characteristic color to the plant. They at all times show a quick reaction to intense light, crowding close to the vertical cell walls after a few minutes of brilliant illumination. The oil globules persist for a short time only, and may not be seen after the first division of the prothallial cell.

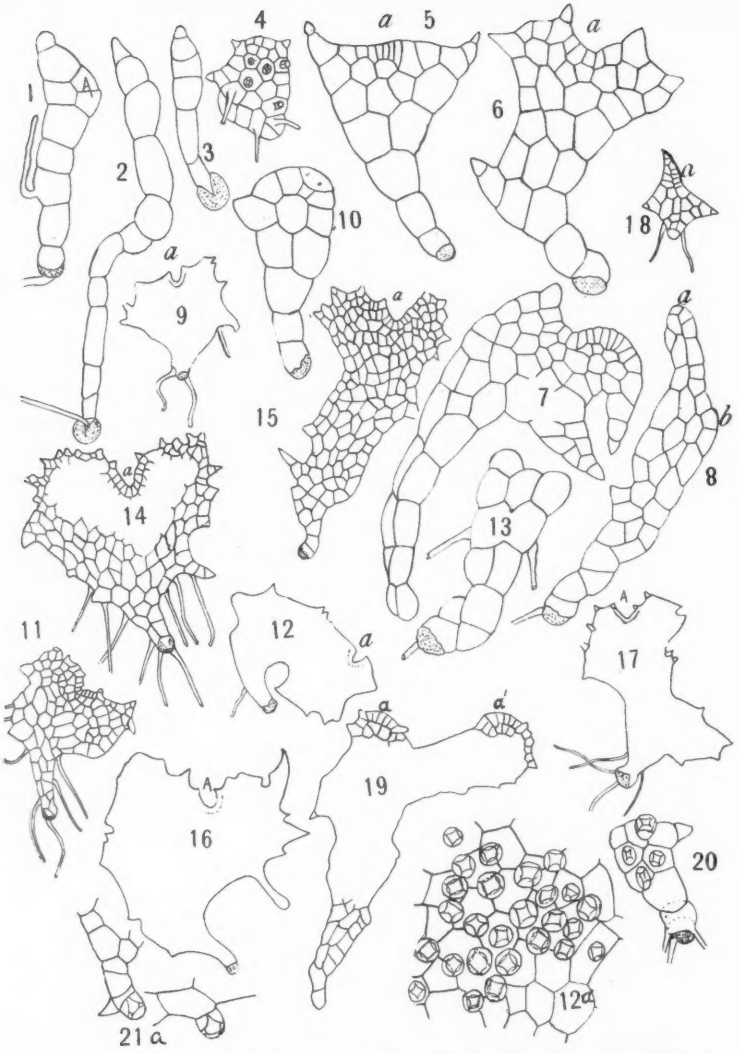
Early development of the prothallia

As was stated above, the first division of the cell may produce two similar cells, or may cut off a small cell which develops into a rhizoid. If the first division produces a rhizoid, the second division produces two cells similar in form and containing chloroplasts. Later divisions of the distal cell may occur in either a transverse

or longitudinal plane. In some instances consecutive transverse divisions occur until a long, protonema-like filament of many cells is formed (figs. 1, 2, 3, 61). The length of the filament may vary, but whether it be one cell or more than one cell in length, it finally produces, by longitudinal and further transverse divisions of its newer cells, a flat plate one cell in thickness. It should be noted that it is the rule for this plate to be formed by regular promiscuous divisions, without any suggestion of an apical cell or group, although exceptions may be found in unusually long filaments. Not infrequently this plate is formed immediately after the first transverse division of the spore (figs. 4, 18). In other specimens the plate formation begins after a chain of two or more cells is evident (figs. 5, 6, 8, 10, 61, 63). Occasionally a prothallium shows that transverse divisions have been followed or accompanied by longitudinal divisions until a strand two cells wide and as long as the simple protonemal structure mentioned above has been formed (figs. 7, 11, 14). Fig. 13 shows an intermediate form where one cell of a single row has given rise to two by longitudinal division. More rarely a definite strand three cells in width is clearly shown (fig. 19). That these strands, one, two, or three cells in width, are not a part of the regular prothallial plate is indicated by the abrupt beginning of the latter (figs. 5, 6, 7, 11, 14).

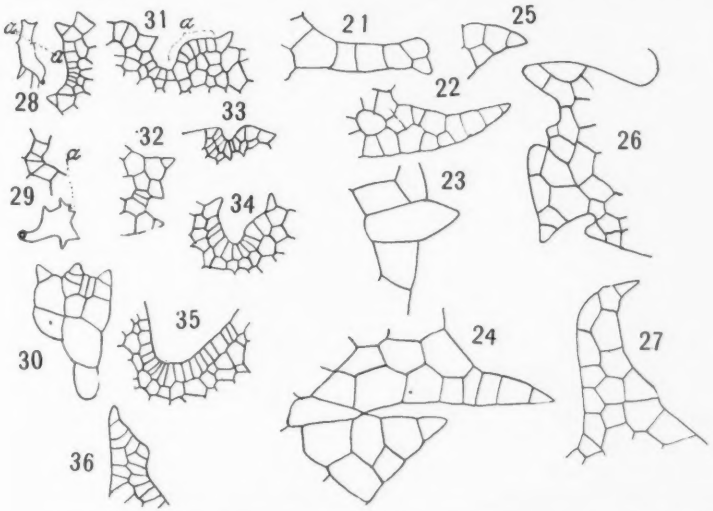
Later growth of the prothallia

The small prothallia increase in size by a promiscuous division of their cells in two planes. The location of growing regions and the direction of division of individual cells seem to follow no general rule. The resulting cell plates lack to a marked degree the regularity and symmetry usually found in the prothallia of related ferns. A glance at figs. 1–20 and at pl. XIII will give an idea of the many and various forms found. This continued promiscuous growth and division of the cells in the body of the prothallium is characteristic of *Camptosorus rhizophyllus*. In many cases prothallia of considerable size are formed by this growth alone before the formation of an apical region, as must have been true in those shown in figs. 15, 19, and 59. This type of growth continues after the appearance



FIGS. 1–21 *a*.—Figs. 1–3, protonema-like prothallia, with possible beginning of apical growth at *A* in fig. 1, ×120; fig. 4, small prothallium with mature antheridia, ×60; figs. 5 and 14, unusually symmetrical growth with distinct apical groups; figs. 6, 7, 8, and 10, examples of unsymmetrical forms, ×120; fig. 13, longitudinal division of one protonemal cell, ×120; fig. 15, prothallium with large plate of cells formed before appearance of apical group, ×40; fig. 19, prothallium with two growing points, ×40; fig. 20, very small prothallium with mature antheridia, ×120; fig. 21*a*, antheridia borne on marginal outgrowths, ×120; fig. 12*a*, group of antheridia from central part of fig. 12, ×120; the other figures show various unsymmetrical arrangements of apical groups.

of the apical region and even after the formation of the archegonial cushion or meristem. It is shown especially in the formation of various marginal outgrowths and in the plicate or crispate form of old prothallia. Such marginal structures, resulting from irregular



FIGS. 21–36.—Figs. 21–27, various marginal outgrowths; fig. 23, one-celled form growing directly from regular margin; see fig. 21*a* for outgrowths bearing antheridia; figs. 28–36, various forms of apical cells and groups; fig. 36 is *a* of fig. 18 more highly magnified; fig. 28, wholly lateral apical group, and fig. 31, apical group (*a*) beside a sinus; all ×120 except small figures in figs. 28 and 29.

cell multiplication having no connection with apical growth, are also characteristic of *Camptosorus*. These marginal structures may be of one or of very few cells (figs. 6, 17, 56, 61), or may be of many cells and approach the dignity of lobes (figs. 7, 16, 58). The form of several such lobes is shown in figs. 21–27.

The region of apical growth

Although the prothallia may attain considerable size and even mature gametes without the appearance of a typical V-form apical cell or a distinct apical group, such a growing region is quite usual and typical. The appearance of the apical cell or group is

varied as to both time and position. In long, protonema-like structures the beginning of a specialized growth region is usually indicated by the division of a cell once or twice removed from the distal end (fig. 1). In case a plate of cells has been formed as mentioned above, a group of cells anywhere about the periphery of this plate may become more active than the neighboring cells, and thus constitute an apical region or group. The formation of a distinct, individual, V-shaped apical cell has not been noted, although forms like figs. 8, 29, 30, and 32 suggest such. In the plant shown in fig. 8 the growing region would probably appear between *a* and *b* rather than as a result of any activity of the cell at *a*. In many instances the apical region is quite symmetrically placed (fig. 14); but an examination of figs. 7, 12, and 17 will show the extreme variation of position, and how far from symmetrical the placing of an apical group may be. Fig. 19 shows an even more interesting case, of which a few have been found, where two such regions have been

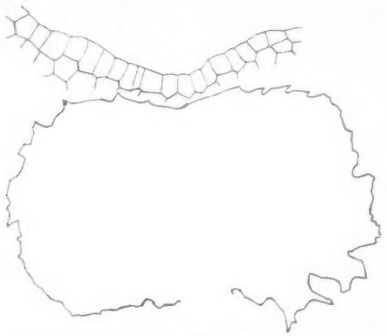


FIG. 37.—Large prothallium with distal margin almost plane as a result of activity of large apical group; ×28 and 160.

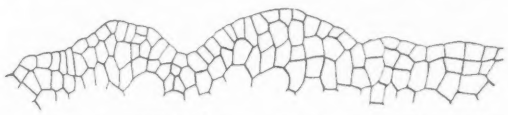


FIG. 38.—Portion of margin of large prothallium, showing almost continuous growing group; ×160.

formed. The very striking irregularity in the formation of the apical group is further shown in figs. 28–36 and in figs. 56 and 57. Fig. 28 with the group (*a*) entirely lateral to the evident axis of elongation, fig. 36 (an enlargement of *a* in fig. 18), and fig. 31 with the apical group beside a sinus are worthy of notice. Figs. 33, 34,

and 35 show about as regular or symmetrical a formation as has been found.

Another point to be noted is that as the prothallia grow older the apical group increases its activity, with the result that instead of being in a sinus of considerable depth it is pushed outward to form an almost straight margin at that place (fig. 37). Closely related to this phenomenon are the cases of proliferation now to be mentioned.

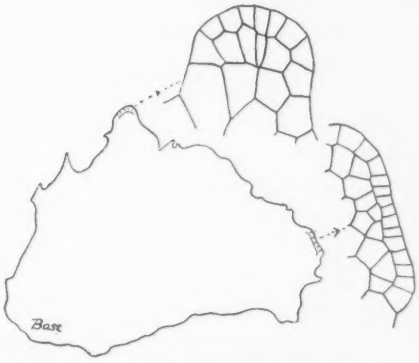


FIG. 39.—Old prothallium that has developed two special growing regions; ×28 and 160.

Prothallia which have grown for four or five months, and those which have been allowed to become quite dry and recover, sometimes continue irregular growth at one or more points on the margin. Fig. 38 shows a part of the margin of a large prothallium with renewed growth, after desiccation for several days. Fig. 39 shows two such regions on one prothallium. Fig. 40 shows an advanced development of such a region that has produced a rhizoid and might continue growth independent of the original prothallium. On some such proliferations antheridia are formed regularly and abundantly. The presence of archegonia has not yet been noted on them.

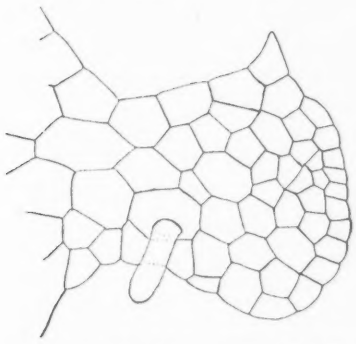


FIG. 40.—Special growing point similar to those in fig. 39, but more fully developed and showing rhizoid; ×160.

Size of prothallia

Prothallia bearing antheridia vary much in size at the time of maturing their first sperms. Examples of plants composed of but few cells and with mature antheridia (figs. 4, 20) are not uncommon; on the other hand, many reach a width of 1–2 mm. before producing their first antheridia. The dwarf antheridial plants never attain any great size; they are but one cell in thickness, and never produce archegonia. Fig. 41 shows the prothallium in fig. 20 and another taken at the same time from the same part of a culture and drawn to the same scale. These were just maturing their first antheridia. Antheridia are quite evenly distributed over the lower surface of the older portions of the large prothallia, and over the lower surface and margins of the dwarf forms (figs. 4, 12*a*, 20). Fig. 12*a* shows the abundance of antheridia on the large plant in fig. 12. Occasionally antheridia appear on the margins and even on the marginal outgrowths of large prothallia (fig. 21*a*). Five months old prothallia, bearing both antheridia and archegonia, are sometimes 4 mm. wide. They are strictly dorsiventral, although they show a marked tendency to take an upright position. The margins are distinctly crisped and plicate. After six to eight months, the marginal growth of the plants continues and the older portions die away much as in the liverworts.

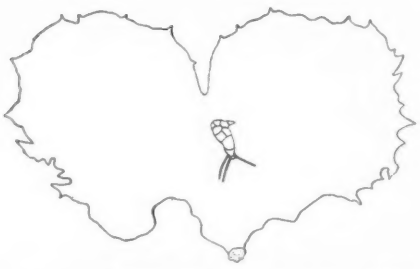


FIG. 41.—Two prothallia of same age (as indicated by maturity of first antheridia) and from same bit of soil; ×28.

The rhizoids develop regularly from the lower surface of the prothallia, and are most abundant on the central part of the older portions, that is, at the base of the plant. They are long, slender, and sparingly branched near their free ends. The photographs reproduced in pl. XIII give a general idea of their character. The only point worthy of note is that mentioned above in connection

with the germination of the spore, namely, that the first division of the cell may produce a second prothallial cell instead of a rhizoid.

Antheridia and archegonia

The antheridia develop in much the same general way as described by CAMPBELL[1] for *Onoclea Struthiopteris* as a type of the Leptosporangiatae. The stages in development are suggested in figs. 42–46. The one point worthy of mention is the formation of the neck cell. This cell is cut off by a division of the initial antheridial cell in a plane parallel to the prothallial surface, and is very regularly found, although sometimes the more orthodox development shown in fig. 44 is evident.

The archegonial meristem is but little in evidence, being of small area and not conspicuous. It develops on prothallia soon after the appearance of the antheridia, and is found only on such plants as have a well formed apical group. Archegonia are found on plants 1.5 mm. or more in width. Their development is in every way typical of the Leptosporangiatae, but only a few (4–8) are found on a prothallium.

Summary

The spores of *Camptosorus rhizophyllus* germinate very irregularly in point of time.

Prothallia bearing antheridia only and those bearing both antheridia and archegonia are produced.

Both antheridial and archegonial prothallia show a wide variation in size and form, the result of a promiscuous cell division and growth.

The formation of a typical V-shaped apical cell is rarely found, if at all, and the apical group is usually unsymmetrically placed.

Old prothallia, bearing both antheridia and archegonia, may develop several marginal growing regions, and may even produce proliferations capable of independent growth.

The archegonia follow the typical Leptosporangiatae in their

[1] Mosses and ferns, 1905, p. 315.

development. The antheridia usually form a neck cell before the regular antheridial divisions.

INDIANA UNIVERSITY
BLOOMINGTON, IND.

EXPLANATION OF PLATES XII AND XIII

(Figs. 1–41 in text)

PLATE XII

All figures ×660

FIGS. 42–46.—Stages in development of antheridia; fig. 44 shows orthodox form sometimes found, the others show more usual form with a neck cell.

FIG. 47.—Fresh, mature spore.

FIG. 48.—Spore with cell showing through ruptured coats.

FIG. 49.—Spore with rhizoid showing first.

FIGS. 50, 51.—Rhizoids issuing from the cell exposed by the rupture of the coats: *og*, oil globules.

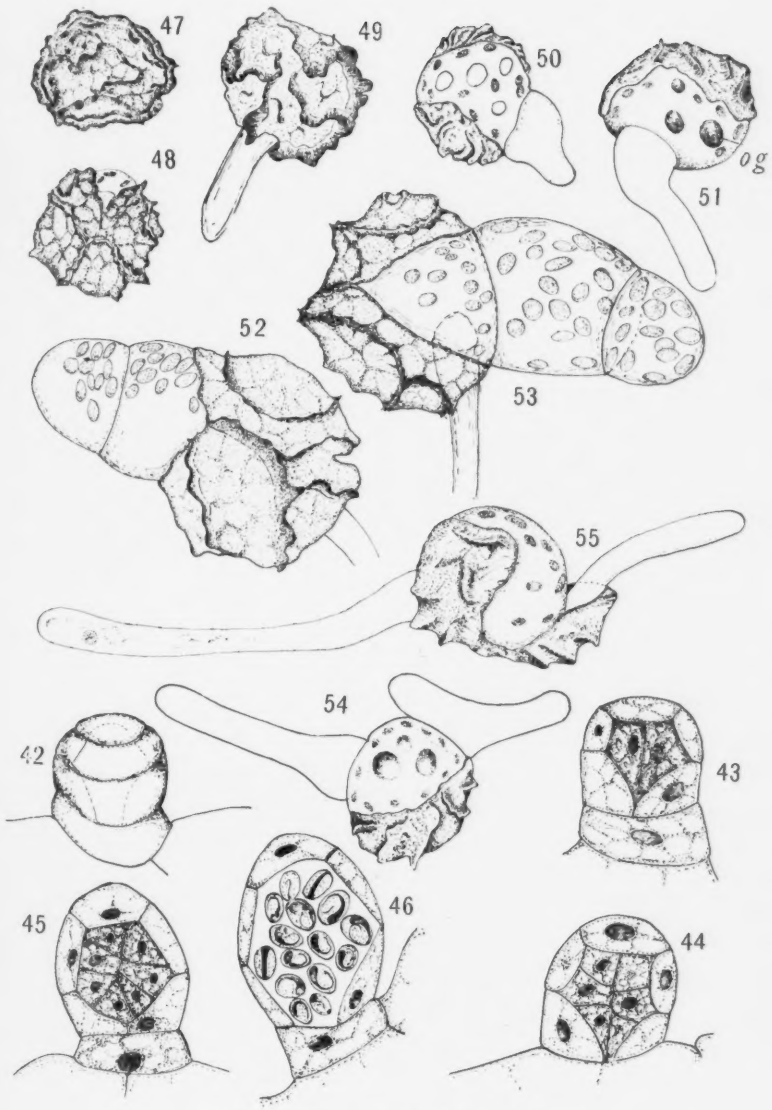
FIGS. 52, 53.—First and second divisions of the prothallial cell.

FIGS. 54, 55.—Two rhizoids formed by the undivided spore.

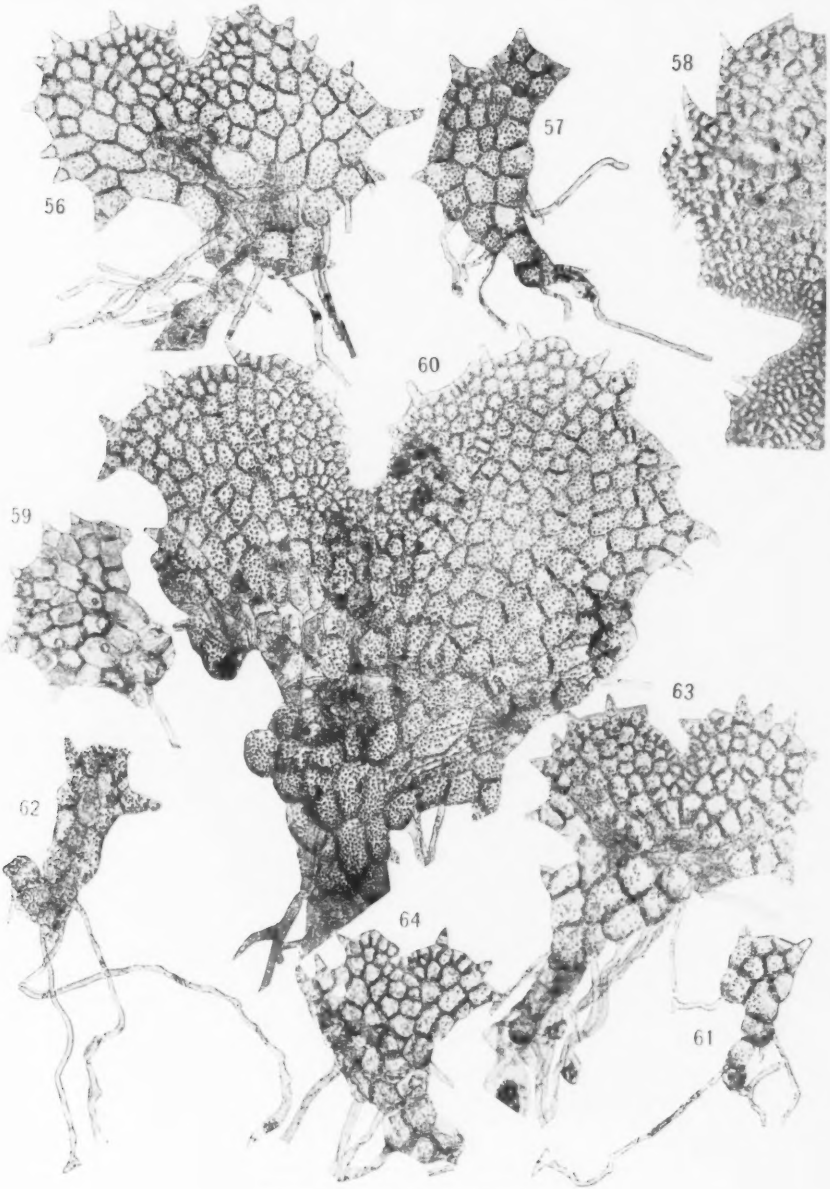
PLATE XIII

All figures ×75

FIGS. 56–64.—Photographs of living prothallia which, with many others, were taken at one time from one small bit of soil; only enough are shown to indicate the variation in form and growth.



PICKETT on CAMPTOSORUS



PICKETT on CAMPTOSORUS

CURRENT LITERATURE

BOOK REVIEWS

Genetics

Some one once said, perhaps more epigrammatically than truthfully, "the progress of a science is in direct proportion to the mathematics used in its development." Whether generally true or not, the constant and rapid progress of genetics since the introduction of Mendel's mathematical notation is a great argument in favor of the statement. At the same time, the chaos that can result from the unwarranted use of mathematics without other premise or analysis is only too familiar to biologists. It has seemed as if those best trained in mathematics were the first to forget that their science is merely a shorthand method of stating the facts, that no more can come out than goes into the mill, though it should come out in a shape more conducive to thorough mental digestion. The slogan of certain biometricians, "there are no premises, all is treatment," has brought many biologists to that state of mind in which they could take seriously POE's sly dig in the "Purloined Letter." In speaking of the necessity of putting oneself in the mental attitude of the thief if the hiding place of the stolen letter were to be discovered, he says: "As poet *and* mathematician, he (the thief) would reason well; as mere mathematician he could not have reasoned at all."

It remained for JOHANNSEN to prove that he is poet, biologist, and mathematician, by showing some four years ago the true relation of KARL PEARSON'S beautiful developments of mathematical methods to genetic research. The motto through the whole 25 chapters of his 500-page book was: "Wir müssen die Erblichkeitslehre mit Mathematik, nicht aber als Mathematik treiben!" JOHANNSEN'S work on the comparative permanence of homozygous types published under the title *Ueber Erblichkeit in Populationen und in reinen Linien* (1903) had already been enthusiastically received by many investigators, partly by reason of the author's mastery of a persuasive style and partly because the conclusions fitted data with which his readers were personally familiar. For these reasons, this elaboration of his ideas met with a cordial reception that is not the fate of many textbooks. But one unfavorable criticism of any importance could be made. The author did not treat adequately the numerous genetic researches in which the problems of heredity had been attacked by methods unlike his own. There is no hesitancy, therefore, in saying that the new edition,[1] with its 30 chapters and 722 pages, to which this criticism may not

[1] JOHANNSEN, W., Elemente der exakten Erblichkeitslehre. Zweite Auflage. 8vo. pp. xi+723. *figs.* 33. Jena: Gustav Fischer. 1913.

be applied with justice (if one excepts cytological research), will be a welcome addition to genetic literature.

In its present form, the work might very easily be divided into two books with separate titles that could be used independently. The one is a thorough introduction to statistical methods as they should be used in the service of biology; the other is a well balanced discussion of the present status of genetic conceptions.

As might be expected, it has been the general discussion of heredity that has received the bulk of the revision; the chapters on biometry were admirably done in the first edition, and the static nature of their substance was such that little change has been necessary. Scarcely a word has been altered in the first five chapters, though CHARLIER'S short method for determining the standard deviation has been added. In chapter 6 the discussion of mean error has been revised and a demonstration from the domain of plant physiology has been added. From this point to chapter 22, only chapters 12 and 13 are new, but the remainder of the book is entirely as written.

In chapter 12 the more recent investigations concerning the possible effect of selection on pure lines are described, while in the next chapter the "misunderstandings" of certain authors who have opposed the theory of permanence of homozygous types are taken up and disposed of with very clear logic, though the style of the rejoinder is sometimes a little caustic.

The last seven chapters of the book are so crowded with information that only a hint as to their contents can be given. They must be read by all who are interested in genetics. Sixty pages are given up to the influence of the factors of environment on variation and 160 pages to Mendelism in its various phases, including heterozygosis, inbreeding, sterility, coupling, and sex determination. Mutations are considered rather concisely in the next to the last chapter, the author being rather of the opinion that the peculiar behavior of *Oenothera Lamarckiana* will ultimately be shown to be the result of segregation and recombination, as has been suggested recently by HERIBERT-NILSSON. The final chapter is a résumé, with observations on eugenics, race hygiene, and evolution.

With reference to the position taken in his earlier work concerning the action of selection, the author remains as firm as a rock. He adds further data of his own to support his position and shows very clearly that the seemingly opposing conclusions of various investigators either are due to fallacious reasoning or are based upon material that is not easily divested of complications that confuse the main issue. To critics who deal only with generalities he makes the following reply that may well be taken to heart by those who deal with evolution from an easy chair:

> Man hat mich kurzsichtig genannt, in Bezug auf die Selektion. Ich konstatiere dies mit Vergnügen; die Prämissen einer oft maszlosen spekulativen Fernsichtigkeit waren ja gerade zu untersuchen und würden wertlos gefunden.

It will doubtless surprise many that JOHANNSEN maintains a firm Lamarckian attitude throughout his book, dealing particularly sympathetically with the work of SEMON. He says: "Man hat mich ferner 'reiner Weismannianer' genannt. Jeder solche 'man' hat mein Buch nicht gelesen oder nicht verstanden." The reviewer must admit, therefore, that he has not understood the author, for after reading the volume he is still firmly convinced that in its essentials it is more nearly Weismannian than Lamarckian. Of course he would not accuse the author of maintaining the morphological hypotheses of WEISMANN with the biophores, determinants, and ids all built into a beautiful structure, but the germ-to-germ inheritance, the dependence of transmissible qualities upon germinal constitution, the invalidity of any particular assumption as to breeding power from the appearance of the soma, and the comparative freedom of the germinal substance from the influence of ordinary environmental changes, as maintained throughout the work, will be classed by most biologists as belonging rightly within the scope of WEISMANN'S conception of heredity.

Very few new terms are introduced by JOHANNSEN in this edition of his book, but two have appeared that seem justified in spite of the abuse that has been showered on the roots used. Individuals that belong to the same phenotype are "isophenous"; individuals that belong to the same genotype are "isogenous." In addition he has adopted WEBBER'S term "clone" for a bud individual.

Taken all in all, one must be very critical to have anything but praise for the new *Erblichkeitslehre*, and it is confidently predicted that it will long remain a classic.—E. M. EAST.

MINOR NOTICES

North American Flora.[2]—Volume 15, part 1, contains the Sphagnaceae by ALBERT LE ROY ANDREWS, the Andreaeaceae by ELIZABETH GERTRUDE BRITTON and JULIA TITUS EMERSON, and the Archidiaceae, Bruchiaceae, Ditrichaceae, Bryoxiphiaceae, and Seligeriaceae by ELIZABETH GERTRUDE BRITTON; part 2 contains the Dicranaceae and Leucobryaceae by ROBERT STATHAM WILLIAMS. New combinations occur in *Sphagnum*, *Ditrichum*, *Dicranella*, *Campylopodium*, *Oncophorus*, *Austinella*, *Leucoloma*, and *Dicranodontium*. New species are described in the following genera: *Dicranella* (2), *Dicranum* (1), *Campylopus* (4), and *Octoblepharum* (1). Volume 22, part 5, is devoted to a continuation of the Rosaceae by PER AXEL RYDBERG and contains the genera *Poterium* to *Rubus* inclusive. New species are described in the following genera: *Agrimonia* (2), *Adenostoma* (1), *Geum* (4), *Sieversia* (1), *Cowania* (1), *Cercocarpus* (7), and *Rubus* (19).—J. M. GREENMAN.

[2] North American Flora. Vol. 15, part 1, pp. 1–75, June 14, 1913; part 2, pp. 77–166, August 8, 1913. Vol. 22, part 5, pp. 389–480, December 23, 1913. The New York Botanical Garden.

Fossil plants.—PELOURDE[3] has contributed the first published volume to the "Bibliothèque de paléontologie," which in turn is one of the 40 divisions of the "Encyclopédie scientifique" under the general direction of TOULOUSE. Under Paleontology, 15 volumes are projected, 3 of which are to be on Paleobotany. The two others will deal with gymnosperms and angiosperms.

The present volume is a compact summary of our knowledge of fossil cryptogams, all but 22 pages being given to pteridophytes. The completeness of the summary may be judged by the fact that the bibliography includes 256 titles.—J. M. C.

Identification of trees.—In order to meet the demands of teachers for a serviceable key for the identification of trees in their winter condition, the authors of *Trees in winter* have reprinted that portion of the volume containing the keys to genera and species.[4] As indicated in the review of the original volume,[5] these keys are based upon the bud, leaf-scar, twig, and occasionally upon the fruit characters. It is anticipated that the convenience of the key in a separate form will be appreciated as an important addition to the equipment for the winter study of our tree flora.—GEO. D. FULLER.

NOTES FOR STUDENTS

Self-sterility.—CORRENS[6] has recently made the phenomena of self-sterility in plants the basis for a searching genetic investigation. After some preliminary experimentation, *Cardamine pratensis* was selected as the material best suited to his purpose, especially as some light had already been thrown upon self-sterility in this species by the investigations of JOST and HILDEBRAND. CORRENS began his study with two specimens of *Cardamine pratensis*, which though derived from the same source (Munster Botanic Gardens) differed markedly in many characters, and were both self-sterile. These two plants (for convenience labeled *B* and *G*) were crossed reciprocally. The offspring, 60 in number, were tested out individually for self-sterility by pollinations (1) from the parents, (2) on the parents, and (3) from sisters. The results are given in such great detail and with such a large amount of easily followed tabular data, that no critic of modern genetic experimental work can criticize the evidence presented on the ground that the details are not all given, or that

[3] PELOURDE, FERNAND, Paléontologic végétale (Cryptogames cellulaires et vasculaires). 16mo. pp. xxviii+360. *figs. 80.* Paris: Octave Doin et Fils. Fr. 5.

[4] BLAKESLEE, A. F., and JARVIS, C. D., The identification of trees. Key to genera and species from "Trees in winter." 8vo. pp. 16. New York: Macmillan & Co. 1913. 30 cts. For sale only by the authors, Storrs, Conn.

[5] BOT. GAZ. **56**:79. 1913.

[6] CORRENS, C., Selbststerilität und Individualstoffe. Biol. Centralbl. **33**:389–423. *pls. I–II.* 1913.

the language is so technical that transmutations must be made before an ordinary biologist can understand it. When the F_1 plants derived from the crosses ($B \times G$ and $G \times B$) were back-pollinated with *B* and *G*, their behavior in reference to seed-setting indicated that they could be separated into four more or less distinct classes: (1) plants fertile with both *B* and *G;* (2) plants sterile with both *B* and *G;* (3) plants fertile with *B*, but sterile with *G;* (4) plants sterile with *B*, but fertile with *G*. Numerically, the 60 F_1 individuals were found to distribute themselves among the four classes in about equal proportions, the actual numbers being 16 *bg*: 16 *bG*: 14 *Bg*: 14 *BG*.

Crosses between the individuals of these four F_1 classes, 720 of which were made, in the main confirmed the results obtained through back-crossing with the parents. Crosses between different F_1 plants and plants obtained from foreign sources all resulted in well-filled capsules. From the facts presented, the conclusion that two inhibitors were operating to present self-fertilization was a most natural and simple interpretation. Accordingly, CORRENS gives to those F_1 plants fertile with both *B* and *G*, the formula *bg*, indicating the absence of both inhibitors; those plants fertile with one parent and sterile with the other are either *Bg* or *bG*, indicating the absence of one and the presence of the other inhibitor; and lastly, those plants sterile with both *B* and *G* are said to be *BG*, indicating the presence of both inhibitors. CORRENS does not dogmatize as to the nature of these inhibitors, except to say that they are hereditary constituents of the germ plasm, which appear to segregate in Mendelian fashion some time prior to the complete development of the egg cells and pollen grains. Hence, self-sterility is not a phenomenon of "Individualstoffe" in the sense in which this term was used by JOST.

In CORRENS' scheme of interpretation, his two original plants are heterozygotes, *B* represented as *Bb* and *G* represented as *Gg*, the letters indicating the presence and absence of two distinct factors for self-sterility. *Bb* gives rise to two kinds of gametes, those with the inhibitor (*B*) and those without it (*b*). *Gg* likewise produces gametes with *G* and gametes from which it is absent (*g*). $Bb \times Gg$ is fertile, but neither $Bb \times Bb$ nor $Gg \times Gg$ would result in seed-formation, as the presence of the factor *B* would inhibit the growth of the pollen from the *Bb* type. The same is true regarding plants of the *Gg* type. The cross $Bb \times Gg$ results in the four types *BG*, *Bg*, *bG*, *bg*, the classes actually obtained as ascertained by the pollination tests. Types *BG*, *Bg*, and *bG* are self-sterile, while type *bg* should be self-fertile. Plants of the *bg* type should be fertile with the other three types; type *bG* is fertile with only *Bg*; type *bG* with only *Bg;* while *BG* is fertile with only *bg*. It is obvious that types *BB* and *GG* could never be formed.

CORRENS' interpretation of his results opens itself to numerous criticisms, for even the most ardent supporter of Mendelian universality would question the actuality of the four classes. Neither the data from the back-pollination experiments nor the data from those in which F_1 sisters were crossed give any notion of clear-cut classes, such as MENDEL secured in his pea work. For

example, $bg \times bg$ should be fertile, but in the tabular data one finds some *bg* plants giving "alle gut," some giving "alle nichts," and some "3 nichts, 3 gut" in crosses, and, so far as the reviewer can ascertain, no *bg* plant was fertile to its own pollen, each attempt invariably resulting in "alle nichts." The testing out of the 60 F_1 plants ($B \times G$, $G \times B$) with the parents gave similar results. Type $bg \times B$ and $\times G$ gave satisfactory evidence of complete fertility in only about a fourth of the cases, the others varying in proportions of "nichts" and "gut" on each plant tested. CORRENS recognizes these difficulties and only advances his interpretation as a crude, but helpful, working hypothesis. He believes there are many different lines of *C. pratensis*, and that these differ much in genotypical constitution, so that the various irregularities whereby his actual data differ from the theoretical expectation are assignable to this cause, that is to say, there were still other inhibitors at work of which he took no notice.

In support of this conclusion, he points out that "keines der 60 Geschwister war einem anderen oder den Eltern völlig gleich"; also, the results secured by crossing these F_1 sisters with the two foreign races. Another complication encountered by CORRENS was the reaction of the same plant toward the same pollen at different times, at one time pollinations resulting in "gut," at other times "nichts," the result possibly of obscure environmental changes.

CORRENS is to be congratulated on again being a pioneer in opening up a new field to a new viewpoint.

COMPTON[7] in two papers has also contributed to the elucidation of self-sterility phenomena. DARWIN'S observations on the existence of self-fertile and self-sterile races of *Reseda odorata* are confirmed, and in crossing experiments undertaken by this author, the following facts were obtained. Self-sterile × self-sterile gave only self-sterile offspring. Certain self-fertile plants when self-fertilized gave only self-fertile offspring; when crossed with self-sterile plants, the same result was obtained. Other self-fertile plants when self-pollinated gave approximately 3 self-fertile to 1 self-sterile offspring; when crossed with self-sterile plants, the proportion of self-sterile to self-fertile offspring is approximately 1:1. COMPTON tentatively regards self-fertility in *Reseda* as a simple Mendelian dominant, self-sterility being recessive.

In his second paper, COMPTON critically reviews the work of MORGAN, JOST, CORRENS, and other earlier investigators of this phenomenon. Until the investigations of these men, the term self-sterility was a veritable "catch-all." The general notion was extant that a self-sterile plant was fertile with the pollen of every plant of that particular species or race, a condition which all three investigators have shown to be untrue. Many records of self-sterility in species rest on faulty observation; in some cases no evidence was at hand to show that

[7] COMPTON, R. H., Preliminary note on the inheritance of self-sterility in *Reseda odorata*. Proc. Cambridge Phil. Soc. **17**:7. 1913.

———, Phenomena and problems of self-sterility. New Phytologist **12**:197–205. 1913.

pollen had ever reached the stigma, or that having reached it, favorable conditions for germination were present. *Laburnum vulgare*, as a case in point, remains self-sterile in the absence of slight mutilations produced by insect visitors. Many examples of species with both self-sterile and self-fertile races or varieties are mentioned. An enormous variation in the degree of self-sterility is noted: at one extreme, self-pollination produces but slightly fewer seeds than cross-pollination; while at the other, a few cases are known in which the stigma and pollen of the same flower are mutually poisonous. Environment produces a marked effect on this phenomenon, as often a change in climate changes self-sterile plants to self-fertile ones. *Biophytum sensitivum* is recorded as self-sterile in its open and self-fertile in its cleistogamous flowers. Our knowledge of causes is exceedingly vague and fragmentary. Examination of stigmas fertilized with their own pollen has shown that although germination takes place, the pollen tube is inhibited in its growth in some way so that it never reaches the embryo sac. In JOST'S experiments no artificial medium was discovered in which pollen tubes would grow their normal length. COMPTON suggests the presence of a soluble diffusible substance in the stigmatic or stylar tissues which acts in a positive manner toward promoting pollen tube growth. An analogy between self-fertility and immunity, and self-fertility and infection is drawn, in line with the suggestive work of JOST, SCHIFF-GIORGIONI, and others. A special section is devoted to a review of the investigations of BAUR, CORRENS, and COMPTON, on the inheritance of self-sterility, and its racial as opposed to its individual nature. Suggestive analogies are also drawn between self-sterility and certain sexual phenomena, such as non-conjugation and conjugation between different strains in certain species of *Mucor*, *Spirogyra*, and *Dasycladus*. The suggestion is made that so-called sex-differentiation in the Mucorineae may be associated with a simple type of self-sterility; homothallic species would lack inhibitors, while heterothallic species might possess two such inhibitory factors. This paper is replete with suggestion and is a review of incalculable value to all students of general biology.—O. E. WHITE.

The wood of Pinus.—GROOM and RUSHTON,[8] in their detailed account of the wood of the five East Indian pines, have kept several objects in view: the affinities of the species, tropical (hydrophytic) or xerophytic features of the wood structure, relationship of the latter to leaf structure, and the nature of the so-called "bars of Sanio." They have devoted the first part of their work to a general statement and discussion, and in the second part have given a detailed description of each species.

P. excelsa and *P. Gerardiana* belong to the HAPLOXYLON section, having single bundles to the leaves, deciduous sheaths on the spurs, tangential pitting

[8] GROOM, PERCY, and RUSHTON, W., Structure of the wood of East Indian species of *Pinus*. Jour. Linn. Soc. Bot. **41**:457–490. *pls. 24, 25*. 1913. GROOM has also given a brief account of the critical identification of the wood of the five East Indian pines in the Indian Forester **39**:409–411. 1913.

on outermost tracheids of the summer wood, and ray tracheids with almost smooth walls. In *P. excelsa* the needles are in fives and the umbo of the cone scale is terminal. The ray pitting of the tracheids, too, is of the large simple type (Grosseiporen). It thus belongs to subsection CEMBRA, and having long cones and thin cone scales belongs to the *Strobus* group. They note that, "as regards width of spring-tracheids, the American species, belonging to the section HAPLOXYLON that most closely approach it, likewise belong to the group *Strobus*," but draw attention to one difference, the thick horizontal walls of the ray cell of *P. excelsa*, which, according to PENHALLOW, is not characteristic of the CEMBRA type. *P. Gerardiana* has a thick cone scale with central umbo, three leaves to the fascicle, and so belongs to the subsection PARACEMBRA of KOEHNE, which has small ray pitting on its tracheids. Like the other haploxylic forms, it has uniseriate tracheary pitting.

The other three species, *P. longifolia*, *P. Khasya*, and *P. Merkusii*, have 3, 3, and 2 needles, respectively, but "agree not only in the diploxylic nature of the leaves, the persistent nature of the sheath of the dwarf shoot, and the possession of a central umbo on the thick cone-scale, but also in that the transition from spring wood to summer wood is sudden (except in *P. Khasya*), the outermost tracheids of the annual ring do not universally bear pits on the tangential walls, the pits on the radial walls of the spring tracheids are often 2-seriate, and the ray tracheids are denticulate."

The authors consider that the pitting of *P. Merkusii* is of extreme interest, showing a transition between that of the cordaitean or araucarian forms and the ordinary abietineous type. The pits are "in one, two, or three rows, or in peculiar nests, of 3 or 4." These nests are surrounded by "Sanio's rims." The similar condition found by SANIO himself in the root of *P. silvestris* seems to have been overlooked, but at least *P. Merkusii* is the only pine so far described with nests in the stem. They compare this cluster pitting to the well known condition described by PENHALLOW in *Cordaites Newberryii*, and to that in *Cedroxylon transiens* of GOTHAN. These clusters of pits correspond to the "starlike" arrangement which GOTHAN considers as intermediate between araucarian and typical abietinean pitting.

With regard to ecological features, they state: "the tracheids are shortest (3 mm.) in the xerophilous *Pinus Gerardiana*, attain a length of 4 mm. in *P. excelsa* and *P. longifolia*, and 4.6 mm. in *P. Khasya*, and the relatively great length of 7 mm. in the tropical *P. Merkusii*. The size of the tracheids is also commented upon, but a separate article dealing with this point is in process of publication. It has a thick cuticle and hypoderma, much transfusion tissue and "a great development of resin ducts," all in excess of *P. excelsa*. Of the other three they state: "as regards leaf structure, all have stomata on all their faces. *P. longifolia*, the most clearly tending to xerophily, has the thickest cuticle (though not so thick as *P. Gerardiana*), and *P. Khasya* has the thinnest. *P. Merkusii* has the most numerous lines of stomata. . . . . In the more xerophilous *P. longifolia* the endodermis has markedly thickened outer walls,

in *P. Merkusii* this is less marked, while in *P. Khasya* there is no indication of such thickening. All three species contrast with the haploxylic Indian species in the feebler development of the tissue separating the endodermis from the vascular tissue."

These structural ecological results are certainly very interesting, and GROOM'S further contribution, which is already in the press, will no doubt add valuable results. It is very desirable that further studies be made on material where the ecological factors are definitely known, and also that a single species be studied under its extreme of wet and dry conditions, in order to determine how much of the change is inherent in the species itself and how much is really due to the external conditions.

The so-called "bars of Sanio" of the Harvard school come in for very severe criticism. They show that these are composed partly at least of pectic compounds, but not of cellulose. They also consider that Miss GERRY mistook SANIO'S description of trabeculae for these structures, and propose the term "Sanio's rims" for them, a terminology which is certainly much more in keeping with SANIO'S idea ("die Umriss des Primordialtüpfels"). Miss GERRY, however, made a much more serious mistake, for she even quotes SANIO'S description of the torus ("diese scheibenförmige Verdickung") as referring to the structures in question.

Resin plates were also found in some of the tracheids adjacent to the rays and also true trabeculae. The authors have also noted the presence of tracheids with bent ends: "when abutting on a medullary ray the end may fork, or bend, so as to run for some distance along the ray and thus form a transition towards a ray tracheid." They also found such tracheids forming radial series apart from the medullary rays.

The detailed description of the species is so arranged that easy reference can be had to any particular feature.—R. B. THOMSON.

Some Jurassic plants.—Among the pteridophytes described by THOMAS[9] from the Marske Quarry of the Middle Jurassic of the Cleveland district of Yorkshire is a new marattiaceous fern, *Marattiopsis anglica*. The genus is "a very common Rhetic and Liassic form, and has been recorded from Sweden, Bornholm, Germany, Poland, and Tongking. Recently two incomplete leaflets from the Jurassic (Kimmeridge) beds of Sutherland have been placed by SEWARD in this genus. Allied forms from the Jurassic of Oregon have been described by LESTER WARD and others under the old name of *Angiopteridium*." Both fertile and infertile pinnae were found. The synangia are considered to have "projected somewhat above the surface, and to have had a fairly firm wall enclosing a number of loculi arranged in two rows; each loculus probably

[9] THOMAS, HUGH HAMSHAW, The fossil flora of the Cleveland District of Yorkshire: I. The flora of the Marske Quarry. Quart. Jour. Geol. Soc. **69**:223–251. *pls. 23–26*. 1913.

formed a projection, and imparted to the synangium a corrugated appearance." The spores are small, about 0.3 mm., and densely covered with fine projections. "The usual tetrad scar is not seen on any example, but a single straight scar instead, which doubtless indicates that the spores were arranged in the spore mother cells bilaterally and not tetrahedrally."

Of the bennettitalean forms, the staminate sporophylls of *Williamsonia spectabilis* Nathorst are "not uncommon at Marske." THOMAS has been able to make an interesting restoration figure of an almost mature staminate flower of this species. The sporophylls are united into a cup below, and probably in the young condition arched over it, straightening out at maturity. The synangia "lie in regular rows, with their long axis at right angles to the sporophylls," and on the upper or inner side. The synangia of each row appear to be borne on slender stalks. "These stalks seem to have been given off on each side of the central portion of the sporophyll, and may be regarded either as lateral lobes of this organ, or possibly as arising as part of a pinnate structure like the microsporophyll of *Bennettites*, which is adnate with the broad structures hitherto termed sporophylls." He concludes, however, that "whatever may have been the method of production of the synangia of *Williamsonia spectabilis*, this form serves (as NATHORST believes) as a valuable connecting link between the type of microsporophyll seen in *Bennettites* (*Cycadeoidea*), where the sporophyll is a reduced pinnate structure, and the *Williamsonia whitbiensis* type, where the sporophyll is undivided, and bears a double row of synangia on its surface." There is evidence also of the presence of the *whitbiensis* type itself in the Marske beds, and of a female strobilus of *Williamsonia* and other bennettitalean remains. THOMAS' study of the leaves is especially interesting, and also his keenness in distinguishing the bennettitalean from the filicinean forms by microscopic examination of the epidermis, etc. Such critical study, which was inaugurated by NATHORST, puts the results from impressions much more nearly on a par with those from the study of petrifactions.

One of the commonest fossil plants at Marske belongs to the Ginkgoales, *Baiera longifolia*, which has not before been recorded in England. THOMAS has not found a complete leaf, but judges that it must be at least 18 cm. in length. By its great size, and also by its epidermal structure, of which three figures are given, it is distinguished from *B. gracilis*, to which the specimens were previously assigned. He also found *Ginkgo digitata* and *Czekanowskia Murrayana* in the Marske beds.

Of the Coniferales two forms were found, *Taxites zamioides*, of which both upper and lower epidermis are described, and *Elatides* (*Pagiophyllum*) *setosa*. Of the latter THOMAS reported, as his article was in process of publication, that "many specimens of this type have been recently found at Roseberry Topping, bearing male and female cones. These seem to indicate the necessity for creating a new species, and probably a new genus for the form here described."

Further study of the fossil flora of this region promises much for our knowledge of the bennettitalean and coniferous forms.—R. B. THOMSON.

Climatic areas of the United States.—In a recent paper, LIVINGSTON[10] has examined the various climatic data made available through the United States Weather Bureau, and finds that among the factors that may be related to plant growth, only precipitation and temperature have been measured with accuracy, and although the distribution of stations is far from ideal, the resulting data are satisfactory. The evaporation data of the Bureau are shown to be extremely meager, being limited to a single year. From the temperature records LIVINGSTON has made a summation of the daily normal temperatures for the days within the frostless season, for a large series of stations, plotted these upon maps of the United States, and drawn isoclimatic lines. Upon these maps he has also drawn isoclimatic lines of (1) the average daily precipitation, (2) the average daily evaporation, and (3) the differences between the precipitation and evaporation for the same frostless period. The results are three maps in which are delimited areas upon the basis of temperature and upon the basis of a water relation. It is possible in this way to characterize climatically the area occupied by any plant or plant community.

In a previous paper[11] the same author has made a chart of the United States from the records of atmometers at 38 stations widely scattered throughout the country for a period of 15 weeks, extending from May to September, 1908. While there is a general agreement between the isoatmic lines and the limits of the great plant formations of the continent, there are certain marked deviations, one of the chief being in the similarity of the evaporation over much of the prairie region and the eastern deciduous forest. This leads the author to conclude that this prairie region is a potential deciduous forest, a conclusion in accord with the success which has attended tree planting within much of this area and with the advance which the forest is at present making upon the prairie, according to GLEASON and other workers. The mesophytism of the northwestern and northeastern conifer forest centers is demonstrated by average weekly rates of evaporation of only about 100 cc. The deciduous forest occupies a region with a weekly summer rate of from 100 cc. to 200 cc., while a similar rate is shown for the southeastern conifer center, which might indicate that this formation is also potentially a deciduous forest. The semi-arid regions of the southwest show an average of from 300 cc. to 400 cc. weekly.

From these and other data it appears that the summer evaporation intensity furnishes a climatic criterion which is more promising for vegetational studies than any other available meteorological data. It has the further advantage of being rather easily determined by the porous cup atmometer, and such data would also be of great importance in agricultural as well as in ecological investigations.—GEO. D. FULLER.

[10] LIVINGSTON, B. E., Climatic areas of the United States as related to plant growth. Proc. Amer. Philos. Soc. **52**: 257–275. 1913.

[11] LIVINGSTON, B. E., A study of the relation between summer evaporation intensity and centers of plant distribution in the United States. Plant World **14**: 205–222. 1911.

Cytology of rusts.—In 1912 OLIVE[12] described an intermingling of perennial gametophytic and sporophytic mycelia of *Puccinia obtegens* throughout the host plant. In a new contribution[13] he shows the same condition to hold in two other species, *Puccinia Podophylli* and *Uromyces Glycyrrhizae*. This intermingled growth gives rise to spermagonia, which are followed by aecidiospores and finally by teleutospores in *P. Podophylli*, or by confluent uredosori and teleutosori in *P. obtegens* and *Uromyces Glycyrrhizae*. In the young spring shoots of *P. Podophylli* the order is usually reversed, teleutospores and aecidiospores appearing on the leaf sheaths, and later aecidia and spermagonia on the young leaves.

The mycelium in the leaf sheath is prevailingly binucleate. In the young leaves the uninucleate (gametophytic) mycelium prevails, while in older leaves the binucleate (sporophytic) mycelium becomes predominant. The aecidiospores of *P. Podophylli* and the uredospores of *P. obtegens* and *Uromyces Glycyrrhizae* are all regarded as secondary in origin and thus apogamously derived, arising solely from the binucleate mycelium, as the reviewer[14] formerly pointed out in the first mentioned case. No sexual fusions were found in the young sori in which the mingled gametophytic and sporophytic mycelia occur. The binucleate cells of the sporophyte push in among the uninucleate hyphae of the gametophyte and there form spores directly. OLIVE believes this apogamous condition to be a result of the perennial habit.

The spermatia alone arise from the uninucleate gametophytic mycelium, although binucleate hyphae often invade the immediate neighborhood of the spermagonia. The reviewer, being unaware of this curious mixture of the two kinds of mycelium, in which binucleate hyphae may become predominant, was led to believe the spermatia, like the apogamous aecidiospores, might arise from the binucleate mycelium. The basal cells and spermatia in which he saw more than one nucleus may have been irregularities without particular significance.

Besides the intermingled condition described, two other states of mycelial distribution were observed: (1) an unlimited growth of the perennial sporophytic mycelium alone in *P. obtegens* and *Uromyces Glycyrrhizae*, producing only secondary uredospores and teleutospores in confluent sori, and (2) a localized distribution of the binucleate sporophytic mycelium, giving rise to a sorus of teleutospores in *P. Podophylli*, or in the other two species to the localized "summer generation" or "repeating generation," producing secondary uredospores and teleutospores.—L. W. SHARP.

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[12] OLIVE, E. W., Perennial gametophytic and sporophytic generations in *Puccinia obtegens* (Lk.) Tul. Science **35**:150. 1912.

[13] OLIVE, E. W., Intermingling of perennial sporophytic and gametophytic generations in *Puccinia Podophylli*, *P. obtegens*, and *Uromyces Glycyrrhizae*. Ann. Mycol. **11**:297–311. *pl. 15*. 1913.

[14] SHARP, L. W., Nuclear phenomena in *Puccinia Podophylli*. BOT. GAZ. **51**:463, 464. 1911.

Seedling anatomy of Lupinus.—BECQUEREL[15] has studied the development of the lupine (*L. albus* and *L. luteus*), applying to this plant the "dynamic" method, by which he correlates the diverse results of other botanists and shows how these differences have arisen. He also utilizes his results to combat various current views of the transitional region of the seedling. This "dynamic" method is much in vogue among a group of French botanists, headed by CHAUVEAUD. Instead of taking a seedling of no definite age, plants of all ages and stages in development are studied and compared. This is the method of necessity employed in studying animal development, but has been considered of little importance in plants, because of the slight amount of "making over" of vegetable tissues which is possible. BECQUEREL, however, does claim that a most important change of this nature takes place in the hypocotyl—the *absorption* of the primary wood, which in the young plant comes up very high in the cotyledonary region and in the older plant is not found at all in the same region and only in a vestigial condition at a lower level. This variation in structure has no doubt led, as BECQUEREL claims, to the diverse statements as to the height at which the traces of root structure have been found by different investigators, but that it is due to the absorption of the primary wood and has the phylogenetic significance that BECQUEREL ascribes to it cannot be accepted on the evidence presented. I find no statement to indicate that BECQUEREL has taken into account a very obvious and natural cause for the disintegration of the primary wood. There is a rapid enlargement and elongation of the elements in the hypocotyl, in contrast to the merismatic activity of the more apical parts. In this growth the tracheary elements naturally cannot take part, and in consequence become dissociated and disorganized, their function being assumed by the secondary elements which now appear. Again, BECQUEREL's statement that these elements have disappeared by reason of absorption, as in animal tissues, postulates the presence of an enzyme such as hadromase, and this has not been shown to occur in any plants except the xylophilous fungi.

The objections that BECQUEREL raises to the current views of the transition region between stem and root will lead, no doubt, to clearer ideas and wording of the subject in future texts.—R. B. THOMSON.

Sea-water and the distribution of plants.—The discovery of any efficient means of determining in a quantitative manner the factors limiting the extent and composition of various plant associations must be regarded as an important contribution to ecology. In salt marshes the concentration of salt in the soil water has long been regarded as a limiting factor, and now HARSHBERGER[16] has

[15] BECQUEREL, P., L'ontogénie vasculaire de la plantule du lupin. Ses conséquences pour certaines théories de l'anatomie classique. Bull. Soc. Bot. France **60**: 177–186. *pl. 4. figs. 5.* 1913.

[16] HARSHBERGER, J. W., An hydrometric investigation of the influence of sea-water on the distribution of salt marsh and estuarine plants. Proc. Amer. Phil. Soc. **50**:457–496. *pls. 2. figs. 7.* 1911.

measured this concentration by means of a convenient type of hydrometer, made the necessary corrections for temperature, and has been able to determine the limiting degrees of salinity which the dominant salt marsh plants of the New Jersey coast are able to withstand. Among the species able to grow in water containing over 1 per cent of sodium chloride, *Salicornia herbacea*, *Distichlis spicata*, and *Limonium carolinanum* seem to have the smallest range of accommodation; while *Spartina stricta maritima*, *S. patens*, and *Juncus Gerardi* show the widest limits. *Typha angustifolia* cannot grow in water that approximates a sodium chloride content of 1 per cent, and in much less dilute solutions shows the detrimental effect of the salt. From a large number of determinations, the height of the plants and the size of their spikes are shown to vary inversely with the concentration of the water in which they developed, the optimum condition being entirely fresh water. The paper contains other valuable data and points the way for intensive studies of the vegetation of salt marshes and alkaline soils.

A few notes on the deposits shown in sections of the salt marsh soil indicate a definite succession in the former vegetation similar to that at present in progress, and that there has been a progressive submergence of the marsh either from a change of tidal level, as held by JOHNSON,[17] or from a general subsidence of the entire coast line, as is believed by most investigators.—GEO. D. FULLER.

Queensland ferns.—Dr. F. M. BAILEY, the veteran colonial botanist of Queensland, Australia, has just published an interesting review[18] of DOMIN'S work on Queensland plants, so far as it concerns ferns and fern allies.[19] The new species and varieties, as well as species new to Queensland, are listed, often with descriptions. The notable feature of the review is the almost constant criticism of DOMIN'S new species and varieties. For example, in regard to *Psilotum triquetrum* Sw. var. *fallacinum* Domin, he says that "the distinctions given seem only those of growth and situation"; *Selaginella flabellata* F. v. M. var. *brevispica* Domin "is scarcely worthy of a distinctive name"; *Marattia oreades* Domin "can hardly be separated from that very variable species *M. fraxinea* Sm."; in regard to two new varieties of *Platycerium alcicorne* and one new variety of *P. grande* he says that "it is scarcely advisable to attach names to isolated plants of *Platycerium*, particularly as differences in their growth and form are so often caused by situation." "Finally," says BAILEY, "I think that

[17] JOHNSON, D. W., The supposed recent subsidence of the Massachusetts and New Jersey coasts. Science N.S. **32**:721–723. 1910; also Botanical evidence of coastal subsidence. Science N.S. **33**:300–302. 1911; also BOT. GAZ. **56**:449–468. 1913.

[18] BAILEY, F. M., Contributions to Queensland flora. Bot. Bull. no. 17. pp. 14. 1913.

[19] DOMIN, K., Beiträge zur Flora und Pflanzengeographie Australiens I. Abt. Pteridophyta (Prodromus einer Farnflora Queenslands). Stuttgart. 1913.

had Dr. DOMIN had a longer experience with our ferns, and observed the great diversity of their form and growth, he would have realized that some of his new kinds were but growths of well known species."

Nearly 70 years of experience with plants in the field, much of it with Queensland plants, add to the weight of BAILEY'S comments. At the time of DOMIN'S visit, the present reviewer was collecting morphological material in Queensland, and can heartily agree with the remarks regarding the variability of *Psilotum*, *Marattia*, and *Platycerium*. In *Platycerium*, particularly, the appearance of the plant is so affected by its position on the tree, that isolated plants might be described as new species, if the differences were not so obviously due, in some cases, to merely mechanical causes.—C. J. CHAMBERLAIN.

A new seed genus.—Miss BENSON[20] has made *Conostoma ovale* Williamson the basis of a new form genus, which she calls *Sphaerostoma*. Along with *C. ovale* the doubtful species *C. intermedium* is included as *S. ovale*. In general structure the seed resembles *Lagenostoma*, and is found most frequently without its cupule (or outer integument), from which it doubtless separated when dropped. All of the epidermal cells of the integument are papillate, and at the apex, where the integument is free from the nucellus, they become so elongated as to form a whorl of epidermal crests, the "canopy" being 8-lobed. These crests Miss BENSON calls "frills."

The structure of the lagenostome, however, is quite peculiar. There is the usual central column of nucellar tissue, surrounded by the moatlike pollen chamber which is invested by the epidermis of the nucellus. But the roof of the pollen chamber is modified into what Miss BENSON regards as "an elastically acting mechanism" which definitely closes the pollen chamber after there has been a dehiscence which admits the pollen grains. This elastic mechanism, forming the roof of the pollen chamber, is the epidermis so modified as to resemble in appearance a multiseriate annulus. The conclusion is that in dehiscence this mechanism straightens elastically, admits pollen grains, and then closes the chamber again.

The seeds are provisionally referred to *Heterangium Grievii* on what seems to be excellent evidence, namely constant association, suggestions of actual continuity of ovules with the parent plant, and the evident morphological relationship to *Lagenostoma*.—J. M. C.

Culture of Opuntia.—GRIFFITHS[21] records some very interesting observations upon the behavior of certain species of *Opuntia* under culture. The plants were grown at the government stations at Brownsville and San Antonio,

[20] BENSON, MARGARET J., *Sphaerostoma ovale* (*Conostoma ovale* et *intermedium* Williamson), a Lower Carboniferous ovule from Pettycur, Fifeshire, Scotland. Trans. Roy. Soc. Edinburgh **50**:1–15. *figs. 3. pls. 1, 2.* 1914.

[21] GRIFFITHS, D., Behavior, under cultural conditions, of species of Cacti known as *Opuntia*. U.S. Dept. Agr., Bur. Pl. Ind., Bull. no. 31. pp. 24. *pls. 1–8. fig. 1.* 1913.

Texas, and Chico, California. At each of these places between 600 and 1500 varieties of *Opuntia* were under cultivation. The general purpose of the investigations was economic, but incidentally the behavior of these plants was very suggestive. Spiny plants grown near the coast of Texas mature spines much more slowly than in drier regions. Changes in the environment seem to have more effect on the production of spicules (bristles) than of spines, the evidence indicating that conditions unfavorable to vegetative production stimulate the growth of spicules. An interesting contrast between the conditions in Southern Texas and California is shown by the fact that the former region is favorable for the production of vegetative growth, while California is better adapted to fruit production. It was found that many species are not promising for breeding purposes because of lack of variability; they are very constantly spiny, and spine protection seems to be directly proportional to plant vigor. The power of recovery from the effect of low temperature is remarkable, especially when the succulency of the plant is considered. A very interesting observation was that in many of the species, especially the larger ones, the plants grown from cuttings and those grown from seeds are very different in appearance; the latter are tree-like and the former are headed on the ground without distinct stems.—J. M. C.

Mitosis in Conjugatae.—In agreement with the earlier work of LUTMAN, VAN WISSELINGH[22] finds that the nuclei of *Closterium* show at all stages an essential correspondence with those of higher plants. Division is strictly mitotic, and the chromosomes, more than 60 in number and of various lengths, all come directly from the reticulum, which is composed of but one material. They are not placed in a ring around a central spindle at metaphase, as LAUTERBORN thought, but form a uniform plate of the usual type. VAN WISSELINGH denies the presence of a continuous spirem at prophase and telophase as reported by LUTMAN. The nucleolus is not of the peculiar kind previously described for *Spirogyra;* in *C. Ehrenbergii* it is really an agglomeration of many small nucleoli. No centrosomes were found.

In *Eunotia*,[23] also, the nucleus divides mitotically, as in other diatoms, but well developed chromosomes are not formed. The nuclear reticulum gives rise to small irregular bodies which become arranged in the form of a "nuclear plate" around the characteristic "central spindle" of the diatoms. The nuclear plate divides to form two daughter plates. VAN WISSELINGH'S results here agree with those of KLEBAHN and KARSTEN on other diatoms, but not with those of LAUTERBORN, who found in several species long and well developed chromosomes in both mother and daughter nuclei.

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[22] VAN WISSELINGH, C., Über die Kernstruktur und Kernteilung bei *Closterium*. Siebenter Beitrag zur Kenntnis der Karyokinese. Beih. Bot. Centralbl. **29**[1]:409–432. *pl. 10.* 1913.

[23] VAN WISSELINGH, C., Die Kernteilung bei *Eunotia major* Rabenh. Achter Beitrag zur Kenntnis der Karyokinese. Flora **105**:265–274. *pl. 10.* 1913.

The value of these contributions is unfortunately impaired by the small size and diagrammatic character of the illustrations.—L. W. SHARP.

Morphology of Tetraclinis.—SAXTON[24] has investigated *Tetraclinis articulata*, the "gum sandarach" tree of Morocco and Algeria. He has given an unusually detailed account of microsporogenesis. The mature pollen grain is uninucleate, and approximately three months elapse between pollination and fertilization; while it is 12 months from the first appearance of the strobili to the complete maturity of the seeds. The development of both gametophytes resembles that of other *Cupressus* forms. Wall-formation in the proembryo occurs in passing from the four-nucleate to the eight-nucleate stage, the mature embryo being somewhat variable in the number and arrangement of the cells. More than one tier of cells takes part in suspensor formation. In the mature embryos three, four, and five cotyledons were found. One of the interesting cytological features is the segregation of the chromosomes into two groups in the prophase of the first division of the fertilized egg. This is taken to be an evidence of the continued individuality of male and female chromosomes. The chromosome numbers were found to be 12 and 24. SAXTON is inclined to believe that the *Callitris* group was derived from the *Cupressus* group through some plant resembling *Tetraclinis*. He traces a geographic line of evolution from northern to southern Africa, and thence by means of former antarctic land connection to Australia. This would make *Widdringtonia* the most primitive of the *Callitris* forms.—J. M. C.

The absorption of water by aerial organs.—It is now pretty generally believed that the absorption of water by the aerial organs of vascular plants is rarely a thing of consequence outside of the Bromeliaceae, although it has been known for a long time that flaccid leaves immersed in water recover their turgescence. Experiments made by various investigators have shown that the cell sap of *Salicornia* and other salt marsh plants has an osmotic pressure considerably above that of sea water. Hence the question arose with Miss HALKET as to the possibility of such plants absorbing water when immersed at high tide. It was found that the aerial organs of *Salicornia* plants can absorb water from a 3 per cent solution of sodium chloride, and a larger amount from distilled water.[25] As might be expected, the amount absorbed is greatly increased if the plants are allowed to transpire freely before immersion, without being able to absorb water through the roots. Experiments made on non-halophytic plants under similar conditions resulted in a loss of water rather than in absorption, hence it is concluded that the absorption noted in the salt marsh plants

[24] SAXTON, W. T., Contributions to the life-history of *Tetraclinis articulata* Masters, with some notes on the phylogeny of the Cupressoïdeae and Calltroïdeae. Ann. Botany **27**:577–605. *figs. 9. pls. 44–46.* 1913.

[25] HALKET, ANN C., Some experiments on absorption by the aerial parts of certain salt-marsh plants. New Phytol. **10**:121–139. 1911.

is due to the high osmotic pressure. It is suggested that in these plants absorption by aerial organs (involving both sea water and atmospheric water) may in nature supplement root absorption, although the latter doubtless is the more important.—H. C. COWLES.

Xerophytic fern prothallia.—The very delicate character of fern prothallia in general and their usual development under humid conditions have made it difficult to account for the establishment of various ferns in dry, rocky situations. Some light has recently been shed upon the subject by experimental cultures of the prothallia of *Camptosorus rhizophyllus* by PICKETT.[26] Exposed to conditions of desiccation arranged to simulate as far as possible those of dry limestone ledges under conditions of natural drought, the prothallia showed complete recovery after 34 days, and 25 per cent recovery after 55 days. Under rather more rigorous conditions, only 50 per cent of the prothallia died after 38 days' exposure to conditions of drought that killed all the prothallia of *Onoclea Struthiopteris* in 48 hours; while under the most rigorous aridity in a sulphuric acid desiccator a small proportion of the prothallia survived 4 days' exposure. All these tests go to prove that the drought-resisting character of the prothallia of *Camptosorus* must be a very important factor in the establishment of this fern in its characteristically xerophytic habitats.—GEO. D. FULLER.

Rhodophyceae of the Indian Ocean.—Mrs. WEBER VAN BOSSE[27] has reported upon a collection of Rhodophyceae made in 1905 on the Percy Sladen Trust Expedition to the Indian Ocean. The geographical distribution shows a great resemblance between the algal flora of the Indian Ocean and that of the Malay Archipelago, as well as that of the east coast of Africa. A table shows the locality, bottom, depth, and distribution of each of the 79 species collected, among which there are 18 new species, and a new genus (*Pseudenosiphonia*) of Rhodemelaceae.—J. M. C.

Plant phyla.—BESSEY[28] has revised his *Synopsis of plant phyla*, making many changes in the arrangement of the orders and families. He recognizes 14 phyla, and gives the latest approximate enumeration of the known species of plants as 233,614. These species are distributed among the current large groups as follows: Thallophytes 79,450, of which the Ascomycetes and Basidiomycetes include 64,000; Bryophytes 16,600; Pteridophytes 4,524; Gymnosperms 540; Angiosperms 132,500.—J. M. C.

[26] PICKETT, F. L., Resistance of the prothallia of *Camptosorus rhizophyllus* to desiccation. Bull. Torr. Bot. Club. **40**:641–645. 1913.

[27] WEBER VAN BOSSE, Mrs. A., Marine algae, Rhodophyceae, of the "Sealark" expedition, collected by Mr. J. STANLEY GARDINER. Trans. Linn. Soc. London Bot. II. **8**:105–142. *pls. 12–14.* 1913.

[28] BESSEY, CHARLES E., Revisions of some plant phyla. Univ. Studies **14**[1]: 73. 1914. Lincoln, Nebraska.